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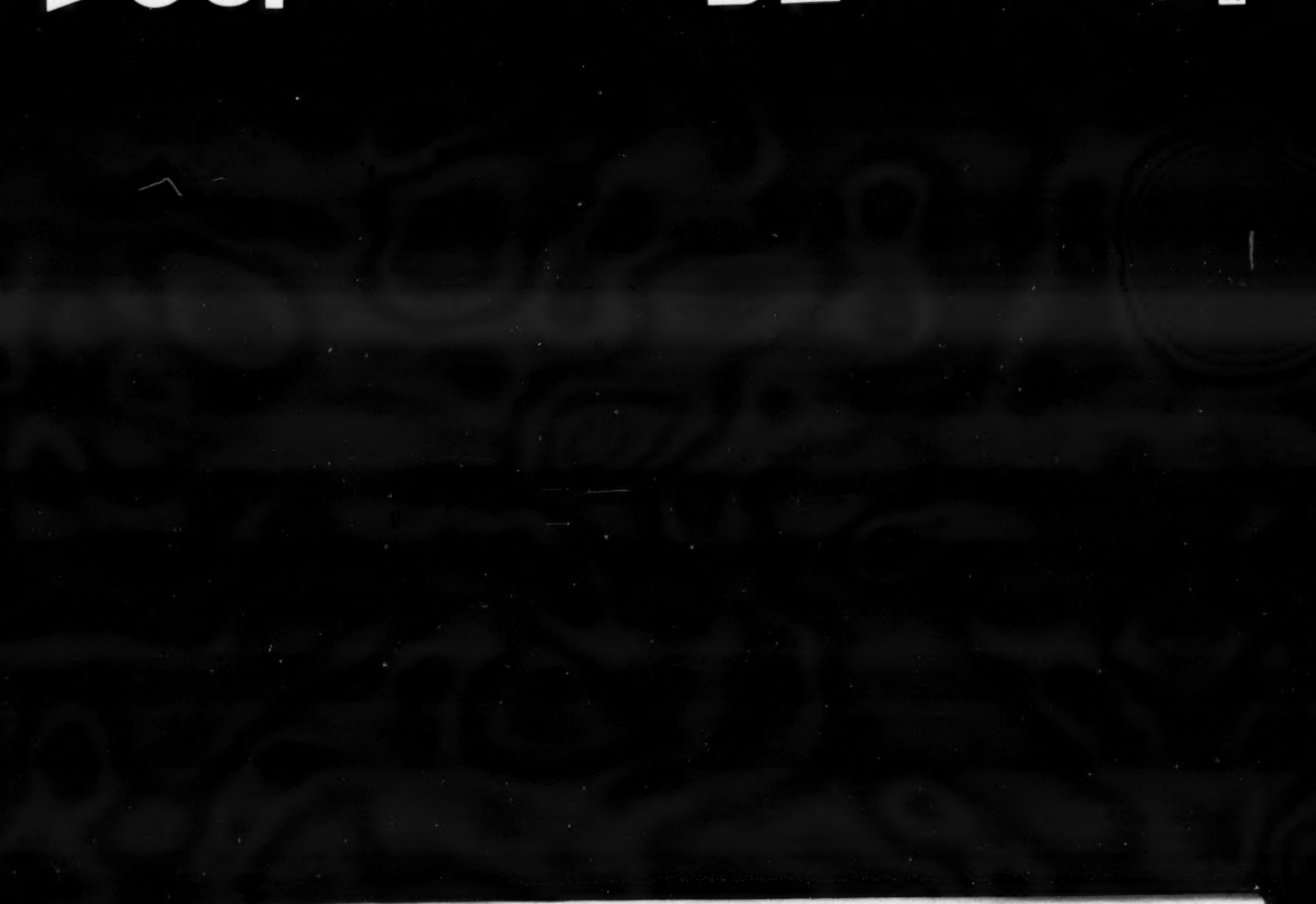

JOURNAL
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THE EARLY DEVELOPMENT OF *HAPLOSCOLOPLOS BUSTORIS* (EISIG)[1]

By Edward C. Horn and C. G. Bookhout

Department of Zoology, Duke University and Duke Marine Laboratory, Beaufort, N. C.

Plates 1–4

INTRODUCTION

The development of members of the polychaete family Orbiniidae (Ariciidae), to which *Haploscoloplos bustoris* belongs, has not been investigated thoroughly. The development of two European species, *Aricia foetida* and *Scoloplos armiger*, which deposit the fertilized eggs in egg cases, has been described to some extent by several authors (reviewed by Thorson, 1946). Since the development of *H. bustoris* appears to be less specialized, however, it offers a better opportunity for comparison with the members of other families. A further purpose of this study is to supply sufficient morphological data of swimming stages to permit their future identification from isolated specimens in plankton studies.

MATERIALS AND METHODS

Haploscoloplos bustoris is the largest of five species of Orbiniids found in the region of Beaufort, North Carolina; it is easily distinguished from the other four by its size and distribution of branchiae (Hartman, 1945). Living in sand or in mixtures of sand and mud, this species is found in greatest numbers at Beaufort at the low tide mark or a few feet below along the edge of sandy shoals. Large, mature worms sieved up off Pivers Island from May through September were returned to the laboratory in jars of sea water where they were washed free of sand and placed in fingerbowls of filtered sea water. Since *Haploscoloplos* never sheds eggs or sperm naturally in the laboratory, the gametes had to be collected artificially. This was accomplished by finely dividing two or three whole males in a petri dish of filtered sea water containing five to seven finely divided females. The eggs and sperm thus liberated were separated from

[1]Aided by a grant from the Duke University Research Council.

the tissue masses by straining the contents of the petri dish through fine silk bolting cloth into a small fingerbowl of filtered sea water. The supernatant fluid was drawn off several times and replaced with fresh filtered sea water. This procedure yielded about 45% cleavage, but development into healthy larvae occurred in approximately only 1% of the total eggs. The removal of all dead eggs and larvae was imperative, while the subculture of healthy larvae seemed helpful.

Diatoms were added to some cultures of older larvae. Other larvae were placed in glass tubes closed at either end with plankton netting and suspended in tidal currents. To still other cultures a variety of sand and mud combinations were offered as substrata for the developing larvae. Despite every attempt to furnish the larvae with what seemed to be an adequate environment none of our cultures survived beyond the thirteenth day. Furthermore, there seemed to be little development beyond the ninth day.

At close intervals larvae and juveniles of various stages were fixed in Bouin's fluid heated to 60°C. Whole mounts were made in oil of cedar after staining in alum cochineal. For setae study unstained larvae were mounted in polyvinyl alcohol and examined under oil immersion.

Preliminary drawings of living larvae were made on squared graph paper with the aid of a Whipple micrometer disc in the eyepiece. All drawings of fixed larvae were made with the aid of a camera lucida.

We wish to thank Mr. Stanley Rhodes and Mr. Leon Hayes for technical assistance. We appreciate the facilities offered by the Duke Marine Laboratory at Beaufort.

OBSERVATIONS

Early Development

The method which was used to obtain fertilization results in uneven development, and, hence, it was not uncommon to find a wide variety of stages at any given time during cleavage.

Twenty minutes after cutting up the worms in sea water a fertilization membrane could be detected on some of the eggs. The first polar body was found 40 minutes after fertilization and the first cleavage one hour later. Two hours and 40 minutes after fertilization 4- to 16-cell stages were present. The eggs, cream colored in the living condition, are very opaque and cleavage is not easily followed. The fertilized egg measures about 160 μ in diameter.

Larval Development

16-hour larva. Thirteen hours after fertilization an early motile larva could be recognized. At 16 hours the typical trochophore larva is positively phototropic for the first time. It possesses a prototroch, a broad continuous band of uniform cilia (fig. 1, proto), and a narrow ciliated band posteriorly, the telotroch (fig. 1, telo); the cilia of the latter are uniform in length except on the dorsal side where they are somewhat shorter (fig. 2, telo). No neurotroch, apical tuft, nor caudal filament could be found. The prototrochal cells appear to be

large and vesicular, but this condition does not exist in the telotrochal region. The gut endoderm (fig. 1, endo) is quite opaque and granular but it already possesses a lumen. A stomodaeum (fig. 2, stomo) is pushing through into the endoderm and probably opens into the gut lumen at this stage. From ventral view a mesodermal sheet (fig. 1, meso) can be seen between the ectoderm and endoderm except in the head region. From side view (fig. 2) it cannot be detected because it has not extended dorsally or ventrally. The ectoderm appears uniform in density except in the region of the prototroch where there are the already mentioned, large, translucent cells.

23-hour larva. From figures 3 and 4 it can be seen that the larva is longer and the mouth opens into the lumen of the gut. In the exact median line a narrow uniformly ciliated band, the neurotroch (fig. 5, neuro) extends from a point just posterior to the mouth to a point just short of the telotroch. At this stage the telotroch consists of cilia of uniform length. A third ring of cilia, the metatroch (figs. 3, 4, 5, and 6, meta), appears for the first time, encircling the larva posterior to the mouth. In addition there are three smaller tufts of cilia equidistant from the metatroch and from each other. They are confined to the ventrolateral surface and are not contiguous with the neurotroch (see figs. 4 and 5, seg).

The bands of mesoderm are prominent laterally (fig. 3, meso) but cannot yet be detected dorsally or ventrally. The ectoderm is lighter than the endoderm and its cells exhibit many mitotic figures. The cells of the anterior end seem to be columnar.

39-hour larva. The period from 23 hours to 39 hours is one of elongation. The 39-hour larva appears more worm-like and less like a larva. The prototroch ventral and anterior to the mouth is composed of one band of cilia, but laterally it splits into two bands (fig. 7, proto a, proto p), one at the level of the cerebral ganglia (fig. 7, cg) and a posterior one at the level of the mouth. The posterior band is incomplete dorsally but the anterior band is complete. There has been no change in the metatroch from the 23-hour stage, but the ventrolateral tufts have now extended further around the larva as segmental bands, leaving only a narrow gap dorsally. In addition three similar segmental bands of cilia have been added posteriorly (fig. 7, seg). Each band is located on what will be the center of a body segment. Thus, the peristomium in the vicinity of the mouth represents the first body segment, the metatroch is on the second segment and the six ciliated bands denote segments 3 through 8. The terminal pygidium represents the ninth segment. The neurotroch extends from the second segment posteriorly to the eighth segment. It is composed of cilia uniform in length, but shorter than the cilia in any other ciliated bands of the body. See Table 1 for a summary of the various ciliated bands which appear during larval development.

In fixed preparations large clear cells appear around the periphery in the prostomium and pygidium. There is also a row of clear cells in the epidermis associated with each of the segmental ciliated bands. From optical sections it appears as though the mesodermal bands are solid. The proctodaeum has not

broken through to form an anus. Strands of the circumpharyngeal connectives can be seen running from the cerebral ganglia to either side of the pharynx. On the dorsal surface lateral to the ganglia reddish-brown eye spots can be seen.

40-hour larva. Setae occur at this stage for the first time. They are present as a single pair of notopodial setae on the third and fourth segments, and they apparently appear simultaneously. A pair of ciliated pits or nuchal organs can be seen clearly on the dorso-lateral surface between the two prototrochal bands. The appearance of a seventh ciliated band indicates the addition of another body segment making ten in all. The split into anterior and posterior prototrochal bands has developed almost to the mid-ventral line.

TABLE I

Occurrence of cilia at different larval stages

	16 HRS.	23 HRS.	39 HRS.	40 HRS.	49 HRS.	70 HRS.	3 DAYS, 6 HRS.	3 DAYS, 16 HRS.	4 DAYS, 17 HRS.
Ant.			+	+	+	+	+	+	
PROTOTROCH	+	+							0
Post.			+	+	+	+	+	+	
METATROCH	0	+	+	+	+	+	+	+	0
SEGMENTAL BANDS:									
Seg. 3	0	+	+	+	+	+	+	+	0
Seg. 4	0	+	+	+	+	+	+	+	0
Seg. 5	0	+	+	+	+	+	+	+	0
Seg. 6	0	0	+	+	+	+	+	+	0
Seg. 7	0	0	+	+	+	+	+	+	0
Seg. 8	0	0	+	+	+	+	+	+	0
Seg. 9	0	0	0	+	+	+	+	+	0
TELOTROCH	+	+	+	+	+	+	+	+	0
NEUROTROCH	0	+	+	+	+	+	+	+	0

49-hour larva. The ciliation pattern is the same as at 40 hours, but an additional seta is present in the notopodial bundle of segments three and four. Thus, there are two notopodial capilliform setae per bundle on segments three and four and one notopodial seta on segment five. From a side view (fig. 8) it can be seen that the anterior prototrochal (proto a), metatrochal, and telotrochal bands are the only ones which are complete dorsally; although not shown in figure 8 only the prototroch and telotroch are complete ventrally, the neurotroch interrupting both metatroch and segmental bands.

70-hour larva. The ciliated bands are still present as in the previous stage, but as shown in Table 2 six segments possess setae. All except the last setigerous segment has notopodial and neuropodial bristles. Of the setae in the notopodial bundle two are long and serrate while one is short and appears nonserrate. The same condition exists in the neuropodial setae when three are present, but

when two or one is present they are clearly serrate. All short, nonserrate, capilliform setae will become serrate as they develop.

One pair of dorsal wing-like branchial lobes appears on the tenth segment just anterior to the pygidium. There are also two conical anal cirri present for the first time.

At this stage the mesodermal band can be seen clearly between the gut and ventral epidermis. The beginning of the coelomic cavity can be seen for the first time in the anterior region of the body around the pharynx.

Larva of 3 days, 6 hours. Both anterior and posterior portions of the dorsal prototroch have disappeared, but both bands are present laterally. The anterior ventral part of the prototroch is represented by a few scattered cilia, but the posterior ventral band is still present although reduced from the condition at 70 hours. There have been no changes in the metatroch or in the telotroch. The neurotroch extends from the mouth back to the eighth segment. The ciliated bands from the third to the ninth segments are still well developed. The ninth segment is now setigerous and more setae are found in the notopodial and neuropodial bundles than at 70 hours. See Table 2. This stage is evidently at the onset of metamorphosis.

Larva of 3 days, 16 hours. At this stage there are only a few scattered cilia of the anterior prototrochal band laterally and dorsally, but the posterior band of the prototroch is not reduced laterally. There are no remnants of the prototroch ventrally. The neurotroch is reduced to patches on the third, fourth, fifth, sixth, and seventh segments. The segmental ciliated bands are now confined solely to the ventro-lateral parts of each segment. The metatroch is somewhat reduced, but it is still a complete band, while the telotroch is still well formed. Metamorphosis is nearly complete.

From figure 9 it can be seen that the gut is completely separated from the body wall for the first time. The dorsal and lateral body wall is thin and covered by simple cuboidal epithelium, whereas the ventral wall is thick, preparatory to nerve cord formation. Constrictions can be seen in the body wall which denote the limits of the segments, but septa could not be detected from optical sections.

The cerebral ganglia consist of a pair of large spherical bodies in the prostomium (fig. 9, cg) surrounded by many spherical nuclei. In the ventral portion of the prostomium there are several large vacuolated cells. Similar cells are still present at the posterior end of the pygidium and isolated cells in each segment of the ventral body wall.

Large wing-like branchiae are found on the dorso-lateral border of the tenth segment, and a smaller pair of branchiae appear on the eleventh segment (fig. 9, br2). Cilia along the posterior border of the first pair of branchiae are prominent and comb-like. The anal cirri (fig. 9, ac) at this age are longer and bear a few short terminal cilia.

Optical sections reveal a differentiation of the gut into a mouth, pharynx, esophagus, stomach, intestine, and a terminal anus. The pharynx is in the peristomial or first body segment and is thick-walled. The esophagus is thin-

walled, contorted, and present from the second through the fourth segments. The stomach has a broad lumen and extends from the fifth segment to the seventh. The intestine is thin-walled and extends from the eighth segment to the anus at the end of the pygidium.

Juvenile of 4 days, 17 hours. This stage marks the disappearance of all ciliated bands, hence, it is considered a juvenile, not a larva. The only cilia present are

TABLE 2

Number of setae per segment and their order of appearance

BODY SEGMENT	40 HRS.	49 HRS.	70 HRS.	3 DAYS, 6 HRS.	3 DAYS, 16 HRS.	4 DAYS, 17 HRS.,	7 DAYS
1							
2							
3 Noto.	1	2	3	3	3	4	4
3 Neur.	0	0	3	3	3	3	3
4	1	2	3	3	3	3	4
	0	0	2	3	3	3	3
5	0	1	3	3	3	3	3
	0	0	2	3	3	3	3
6	0	0	3	3	3	3	3
	0	0	2	2	3	3	3
7	0	0	3	3	3	3	3
	0	0	1	1	3	3	3
8	0	0	2	2	2	3	3
	0	0	0	0	1	3	3
9	0	0	0	1	1	2	3
	0	0	0	0	0	1	3
10	0	0	0	0	0	2	2
	0	0	0	0	0	1	2
11			0	0	0	0	0
			0	0	0	0	0

a few scattered cilia of the prototroch anterior to the mouth. As shown in Table 2 there are 8 setigerous segments. Both pairs of branchiae are large and are ciliated along their posterior borders. The coelomic cavity is more extensive, and dorsal to the gut there is a large blood vessel.

Juvenile of 5 days, 17 hours. A third pair of branchiae appear for the first time on the twelfth segment. Septa also are discernible for the first time separating every segment from three to nine. The pharynx and esophagus occupy the same

segments as at 3 days, 16 hours. The stomach occupies one less segment and extends from the fifth to sixth rather than from fifth to seventh segments. The intestine is now divided into two regions; an anterior part which is coiled and extends from the seventh segment through the ninth and a second part with a broader lumen which extends from segment 10 through 12 (see figure 10).

Juvenile of 7 days. The general anatomy of a juvenile at this stage does not differ from the one at 5 days, 17 hours. Two neuropodial setae are now present on the tenth segment, whereas none was present in the previous stage. Two more septa are discernible, occurring between the ninth and tenth segments and between the tenth and eleventh segments. Minor differences exist in the digestive system (see figure 11).

On the posterior border of each segment from 6 through 9 there occurs one pair of dorso-lateral projections believed to be parapodial lobes (fig. 12, pl). They are much smaller than the large branchiae, but they later become progressively larger in each segment after the sixth.

As already mentioned, juveniles maintained beyond this stage showed no marked changes, hence, their descriptions have been omitted.

DISCUSSION

The two members of the family Orbiniidae (Ariciidae) whose development has been described previously demonstrate stages similar in appearance to those of *H. bustoris*, but differ in certain details. Both *Aricia foetida* and *Scoloplos armiger* deposit their eggs in jelly masses; apparently *H. bustoris* does not produce an egg case although these have been looked for at different seasons. The early larvae of *Haploscoloplos* possess in addition to prototroch, metatroch, telotroch, and neurotroch a series of segmental bands of cilia which are not described or figured for *S. armiger* by Cunningham and Ramage (1887) but which are briefly alluded to by Eisig (1914) in his description of *A. foetida*. These accessory bands of cilia are normally correlated with a more prolonged pelagic existence (Spionidae) than the short (about 3½ days) stage exhibited by *Haploscoloplos*. From what is known of *Aricia* this same incongruity appears to exist. The stage figured by Cunningham and Ramage (1887) for *S. armiger* as the most advanced they studied bears a striking resemblance to the latest *Haploscoloplos* we studied except for setigerous segments and gills. *Scoloplos* is figured as possessing 11 setigerous segments and two pairs of branchiae on the next two successive segments; *Haploscoloplos* at a comparable stage possesses 9 setigerous segments and three pairs of branchiae, the first pair of which occurs on the eighth setigerous segment.

The first appearance of dorsal setae in segments 3 and 4 simultaneously (none ever occur in segments 1 and 2 here) correlates well with the first appearance of setae in other polychaete larvae (Wilson, 1932, 1936a and 1936b; Segrove, 1941; Bookhout and Horn, 1949).

It is noteworthy—and as yet inexplicable—that the paired dorsal branchiae of *Haploscoloplos* arise in the 70-day larva on the tenth segment (eighth setigerous segment) and appear later in development on successive segments. A

distinguishing feature of the adult *H. bustoris* is the presence of branchiae no further anterior than the twenty-fourth segment. One might assume that a loss of gills occurs from the tenth through twenty-third segments during the development of the pre-adult; according to Eisig (1914), however, the gills of *Aricia* are present from the tenth segment posteriorly in both the adult and larvae, never more anteriorly. Here then there is no loss of gills during development. In passing it is also interesting to note that gills first appear on the same segment (10) in the larvae of both species.

SUMMARY

1. The development of *Haploscoloplos bustoris* through larval and early juvenile stages was studied from eggs and sperm artificially procured.

2. The early larvae are typical trochophores in that they possess a broad prototroch, a telotroch, and a narrow neurotroch. Later in their development the larvae possess the less typical metatroch and segmental bands of cilia in addition. The swimming stage lasts about three and one-half days when ciliation is lost and metamorphosis is accomplished.

3. Setae appear several days before metamorphosis. A table containing their order of appearance is given. Large, dorsal, wing-like branchiae appear on the tenth segment just before metamorphosis.

4. A brief discussion is given comparing the development of this species with that of the other two members of the family which have been studied.

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EXPLANATION OF PLATES

All drawings were made with the aid of a camera lucida from preserved material; finer details were sketched in. All plates were reduced one-half. Magnifications are for the reduced plates.

PLATE 1

Fig. 1. Optical section of 16-hour larva seen in ventral view. ×210
Fig. 2. Same larva as in fig. 1 seen from right side. ×210
Fig. 3. Optical section of 23-hour larva seen in dorsal view. ×210
Fig. 4. Same larva as in fig. 3 seen from left side and slightly dorsal to show segmental bands of cilia. ×210.
Fig. 5. External appearance of 23-hour larva seen in ventral view. ×210
Fig. 6. Same larva as in fig. 5 seen from left side. ×210

PLATE 2

Fig. 7. Optical section of 39-hour larva seen in dorsal view. ×210
Fig. 8. Optical section of 49-hour larva seen from left side and slightly dorsal to show segmental bands of cilia. ×210.

PLATE 3

Fig. 9. Optical section of 3-day, 16-hour larva seen from right side and slightly dorsal to show segmental bands of cilia. ×210.
Fig. 10. Optical section of contracted 5-day, 17-hour larva seen from left side. ×210

PLATE 4

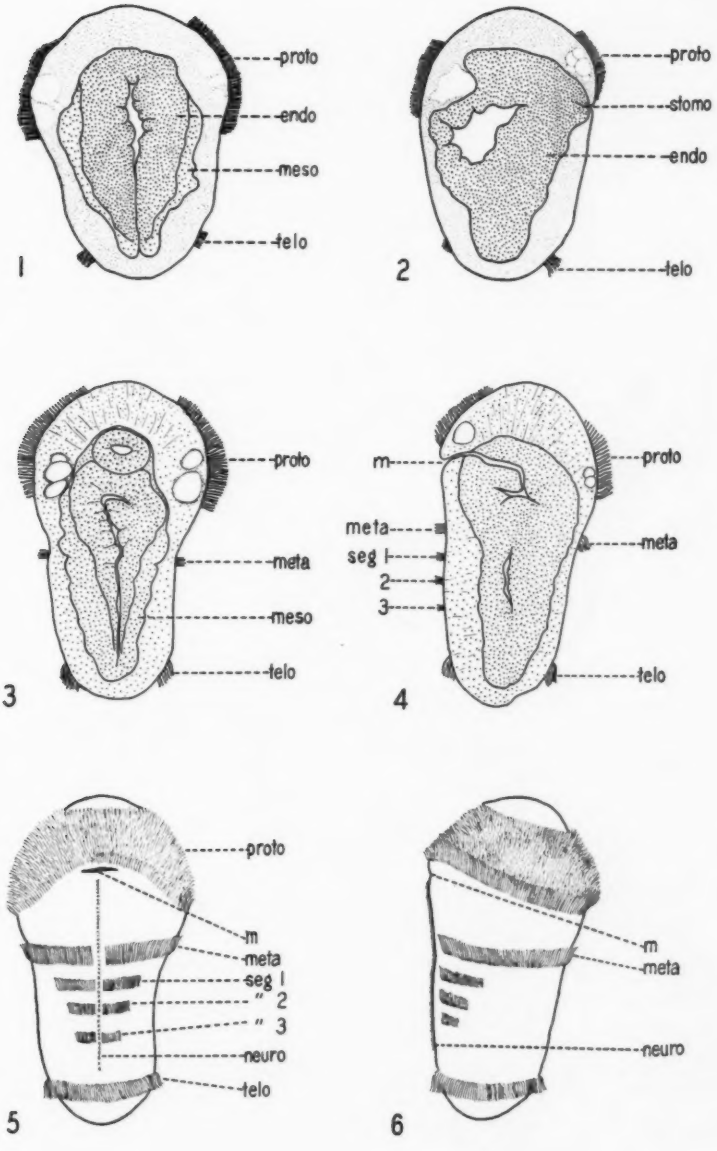
Fig. 11. Optical section of 7-day larva seen in ventral view. ×210
Fig. 12. External appearance of 7-day larva seen in dorsal view. ×190

ABBREVIATIONS

Roman numerals indicate body segments

a, anus
ac, anal cirrus
br 1, 2, etc., branchia
c, coelom
cg, cerebral ganglion
e, esophagus
endo, endoderm
es, eye spot
i, intestine
m, mouth
meso, mesoderm
meta, metatroch
neuro, neurotroch
p, pharynx
pl, parapodial lobe
proto, prototroch
proto a, anterior division of prototroch
proto p, posterior division of prototroch
seg 1, 2, etc., segmental ciliated bands
sep 1, septum 1
st, stomach
stomo, stomodaeum
telo, telotroch

PLATE 1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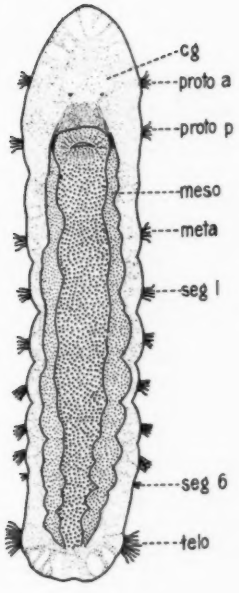
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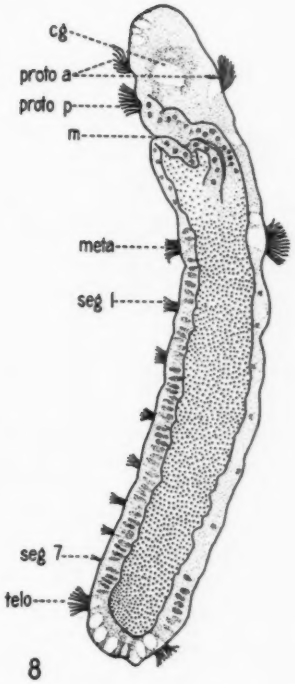
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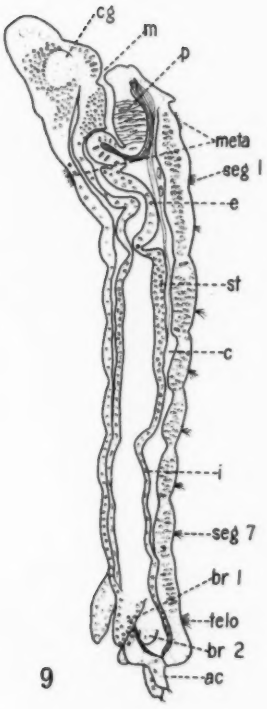
PLATE 3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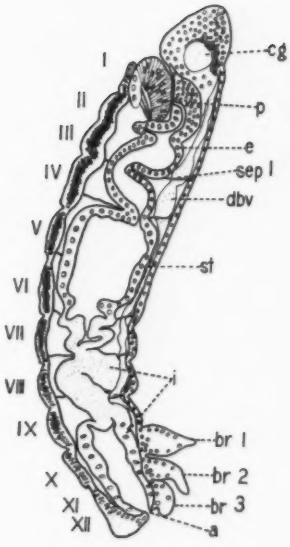
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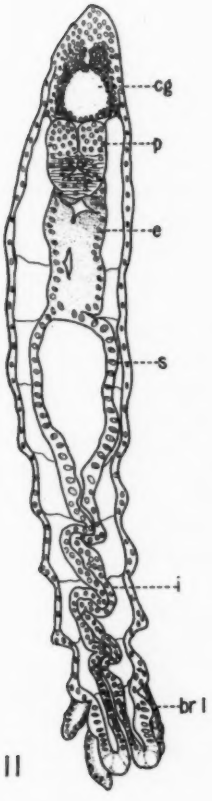
PLATE 4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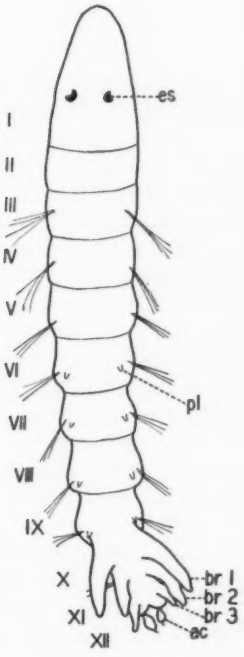
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RECORDS AND DESCRIPTIONS OF DIPLOPODS FROM THE SOUTHERN APPALACHIANS

By Richard L. Hoffman

Highlands Biological Station
Highlands, North Carolina

Plates 5–8

During the month of July 1949, I was fortunate in being able (through the aid of a scholarship from the Highlands Biological Station) to carry out studies on the diplopod fauna of the extreme southeastern Appalachians.

The great majority of collections were made in eastern Macon County, N. C., since a faunal study of the Highlands Plateau was the main objective of the work. However, it was possible to participate in several trips to adjacent localities, and the results thereof may profitably be included in this report.

Insofar as was possible, I made field notes on the ecology of the species obtained, and the more interesting of these are incorporated herein. Although most of the work was of a systematic nature, there seems to be every indication that future collecting will add a number of forms to the known fauna of the Highlands Plateau. Field work in the months of May, June, and October would be especially valuable in this respect.

In addition to recording new localities and ecological notes on established forms, this report includes the description of nine new species, the proposal of a new generic name, and taxonomic notes and changes involving several species.

The following data, condensed from Quarterman and Keever (1947), are pertinent to the collections made in the Highlands area.

"Records of the Highlands Weather Station show an average annual rainfall of 81.73 inches, and average temperatures as follows: June, 64.2; July, 66.5; August, 65.7; annual, 50.5.

"The area contains numerous mountains, both large and small, the slopes of which are usually steep. The south and east sides of most of the mountains are often nearly vertical, granite cliffs with a scanty flora; while the north-facing slopes, although sometimes very steep, show less bare rock and are covered with abundant vegetation. A few of the hills and low mountains have gentle slopes. Many of the small streams that drain the area flow so swiftly that they have washed out deep gorges and formed numerous water falls, varying from gradual cascades over inclined rocks in the stream bed to a sudden drop of many feet, as at Dry Falls.

"There are a number of easily recognized plant communities, with all degrees of transition between them. All undisturbed mesophytic areas are covered with stands of oaks and hickories, the species varying with local conditions. There are a few dead chestnut trees standing, remnants of the time before the chestnut

blight struck in 1927, when the chestnuts held a place with the dominant oaks and hickories.

"Both north and south slopes support an oak-hickory climax. The trees on the north side, however, are larger in diameter and height, and form a more closed canopy than those on the south sides. The understory in the oak-hickory forests is composed of seedlings of oaks and hickories, with many chestnut sprouts, and an abundant growth of laurel, rhododendron, azalea, red maple, cherry birch, and other less numerous small trees and shrubs. The undergrowth on the south slopes is often more dense and thicket-like than on the shaded north-facing sides. Although northern exposures do not show the extreme denseness of vegetation found on the south, many more species are represented in the ground cover there, including ferns, galax, and other shade-loving herbaceous plants.

"There is a distinct type of vegetation along the stream margins and in the small coves formed by the streams. The dominant trees in such places in higher altitudes of the range are invariably hemlock, with some magnolia, red maple, cherry birch, and yellow poplar consistently present, but never attaining the height of the hemlocks. Where the streams are so oriented as to cause a difference in the amount of light received, there is a noticeable difference in the understory plants on the shady and sunny sides. The understory on the shady side is often composed almost entirely of very large rhododendrons forming such a dense shade that only dog hobble, galax, and a few ferns and mosses grow under them. On the sunny sides of the streams, the rhododendron is less dense, and is usually accompanied by laurel, hydrangea, clethra, silver bell, tulip poplar, and seedlings of cherry birch and maple. There is very little hemlock along Big Creek at the lowest altitude of the area. The dominant trees along the stream banks there are red maple, sycamore, and yellow poplar, with the oaks and hickories on the slopes just above."

Since I have discovered from previous experience elsewhere that moist oak-hickory forest contains definite milliped inhabitants, and that certain others are restricted to the hemlock-magnolia "cove forest," most of the collections were made in these two habitat types. Survey collections in other areas—cultivated fields, pastures, etc., and rather dry mountain tops were mostly unproductive (although in Virginia dry exposed mountains often yield large numbers of *Cleidogona!*).

Obviously, comments on the origin and affinities of the Highlands diplopod fauna would be of interest, but considerable supplementary work is prerequisite. At the moment only a few observations may be made in this connection. A most singular situation is the apparently close similarity of the fauna to that of the Great Smokies; this is evidenced particularly by the occurrence of *Howellaria deturkiana* as well as some other species in both regions. It is my understanding that the relationship holds in other animal as well as plant groups.

The occurrence at Highlands of a number of species in groups heretofore restricted to the Southeastern Piedmont is of considerable interest. Good examples are *Onomeris australora* and *Spirostrephon highlandensis* whose closest

relatives occur in central and northern Alabama. The families represented by these two forms (Glomeridae and Lysiopetalidae) are both well represented in Europe and Eurasia.

The majority of the material obtained is retained in my personal collection. All types and the material collected in the Great Smoky Mountains are deposited in the collection of the U. S. National Museum. A series of specimens of the commoner species was presented to the Highlands Biological Station, to be deposited in its Museum of Natural History.

ACKNOWLEDGMENTS

A number of persons have, through their interest and material assistance, contributed much to make my studies the more extensive and thorough. I take pleasure in acknowledging my sincerest gratitude to the following:

Mr. Robert E. Gordon, of the University of Georgia, for making possible trips to several distant places and for the presentation of valuable specimens.

Mr. Fred Bryson, N. C. Forest Service, Franklin, who made possible a trip to the Nantahalas and personally collected material I would not otherwise have found.

Dr. J. Manson Valentine, Highlands, for a number of millipeds taken in connection with his own work on beetles.

Mr. Arthur Stupka, Park Naturalist, Great Smoky Mountains National Park, for his kind cooperation in making it possible for me to collect within the Park boundaries.

Mrs. Margaret Cannon Howell, Highlands, without whose interest in the promotion of research I would not have been able to collect at Tallulah Falls and in the Smokies.

I am especially obligated to Prof. Thelma Howell, Executive Director of the Highlands Biological Station, who deserves much of the credit for the relative completeness of this report.

My debt to my parents is great and long-standing, in connection with this as with most of my other endeavors.

This investigation was made possible by a grant to the Station by the Champion Paper and Fibre Co. of Canton, North Carolina.

Order ONISCOMORPHA

Family *Glomeridae*

Onomeris australora, new species

Plate 5, figs. 1, 2

Type locality.—Reed Creek Falls on Glade Mountain, Rabun County, Georgia (about 5 miles south of Highlands, N. C.).

Type specimens.—Male holotype and four female paratypes, U. S. Nat. Mus. no. 1872; several female paratypes in my personal collection; all collected July 27, 1949.

Diagnosis.—Similar to *O. underwoodi* in most characteristics and possibly

referable to that species. Nineteenth legs of male and female genitalia as illustrated.

Description.—Length, 5.6 mm.; maximum width, 2.5 mm. Dorsum smooth, shining, without fine pubescence.

Seventeenth legs of male three-jointed. Eighteenth legs with joints of about equal thickness, the distal two not abruptly smaller as shown by Loomis (1943: 375) for *Trichomeris sinuata*. Nineteenth legs generally similar to those of *T. sinuata*, differing especially in the large, distally bifid sternal plate bearing a median accessory process, and in the much longer basal processes from the second joints. The third and fourth joints have conspicuous finely corrugated median projections.

Female genitalia attached to sternites of second pair of legs; composed of box-like basal receptacles, each enclosing a set of small valves as figured.

Dorsal color dominantly light brown with a very large oval light blotch on the anterior half on each side of the segments. Ground color darker along middle of back and lateral margins of tergites.

Relationships.—As mentioned above, this form may prove to be the same as *O. underwoodi* Cook (1896: 44), described from Auburn, Alabama. However, the types of *underwoodi* cannot at present be found, and I hazard the description of this montane population on the basis of the unlikelihood that *underwoodi* has such a wide horizontal, vertical, and ecological range. The terminal male legs will doubtless show specific differences when specimens of *underwoodi* become available for study.

Ecology.—At the type locality specimens were found in deep, moist humus in a very dense rhododendron thicket. Immature specimens were abundant near the surface, but adults could be obtained only by going under the loose leaf mold and into the soil itself. Elsewhere I have taken this species principally under large sheets of bark which had fallen from chestnut trees, associated with *Striaria zygoleuca* in particular.

O. underwoodi was found, according to Cook, in rotting leaves and pine needles at Auburn, Alabama. This is of interest inasmuch as very few diplopods are to be found in evergreen woods.

The name *australora* alludes, of course, to the Southern Blue Ridge where the species occurs.

Georgia. Rabun County: Glade Mountain at Reed Creek Falls, six mature specimens taken July 27, 1949.

North Carolina. Macon County: Satulah Mountain at Highlands, many females taken July 20–24, 1949, elevation 4100 feet.

Order COLOBOGNATHA

Family *Polyzoniidae*

Polyzonium bivirgatum (Wood). Proc. Acad. Nat. Sci. Philadelphia, p. 186, 1864.

A single subadult specimen in which the caudal margin of the tergites is nar-

rowly edged with dark brown; taken in company with *Brachycybe petasata* in leaf mold at the base of a beech tree (*Fagus grandifolia*).

Tennessee. Sevier County: along Alum Cave Trail, from Rt. 71, north side of Newfound Gap, Great Smoky Mountains National Park, July 28, 1949.

Family *Andrognathidae*

Brachycybe lecontei Wood. Proc. Acad. Nat. Sci. Philadelphia, p. 187, 1864. Loomis, Proc. U. S. Nat. Mus. **83:** 366, 1936.

This little known species was obtained at two localities, each representing a new state record for North and South Carolina, respectively. *B. lecontei* has been reported from Balsam Gap and Sunburst in the former state, but it is felt that these records actually apply to the following species.

In life, *lecontei* when adult is of a very attractive peach color, the legs somewhat lighter. Immature specimens are white as usual in diplopods.

An adult male, with 47 segments and measuring 16 x 3.7 mm., was discovered on Satulah Mountain near Highlands. It was observed ascending a small tree, about three feet from the ground, and fully exposed to the morning sun.

The immature specimen from Jocassee, with 37 segments and 13 mm. in length, was found in leaf mold under *Kalmia*, on a dry hillside in oak woods.

Originally described from northern Georgia, this species has been definitely reported only from Tallulah Falls in that state (Loomis, *op. cit.*). It is likely that *lecontei* inhabits the southern extremity of the Blue Ridge proper, and that its place is taken further north (*e.g.*, in the Smokies and Balsams) by *B. petasata*.

North Carolina. Macon County: east side Satulah Mountain at Highlands, July 23, 1949.
South Carolina. Oconee County: Jocassee, July 14, 1949.

Brachycybe petasata Loomis. Proc. U. S. Nat. Mus. **83:** 365, 1936.

Two immature specimens, with 30 and 38 segments, were found in company with *Polyzonium bivirgatum* in moist but loose leaf mold at the base of a beech tree, July 28.

The color, not heretofore known, is very distinctive from that of *lecontei* (*q.v.*). "Color in life very bright pink with a vermilion cast, dorsally and on undersides of keels; legs white; head, antennae, and collum yellowish gray."

It would seem that members of the genus *Brachycybe* have a definite predilection for beech woods. These trees were present at the Highlands station for *lecontei;* and a number of specimens of an undescribed species taken in West Virginia are noted to have been found crawling on logs in beech woods! This association is certainly worthy of investigation by ecologists.

The following locality does not add to our knowledge of the distribution of the species, which has previously been found at Chimneys Camp in the Park (Loomis, 1943: 376).

Tennessee. Sevier County: along Alum Cave Trail, from Rt. 71, on north side of Newfound Gap, Great Smoky Mountains National Park, July 28, 1949.

Order CHORDEUMIDA[1]

Family *Striariidae*

Striaria zygoleuca, new species

Plate 5, fig. 3

Type locality.—Satulah Mountain, Highlands, Macon County, North Carolina, elevation 4100 feet.

Type specimens.—Male holotype and female allotype, U. S. Nat. Mus. no. 1873; eight topoparatypes of both sexes in my personal collection and one in the Highlands Museum.

Diagnosis —Distinct from all other members of the genus and family in the white color of the enlarged first segment. The terminal modifications of the male gonopod appear to be of specific value in diagnosis.

Description.—Length approximately 10 mm. as in other eastern members of the genus.

Agreeing in structural characters with *S. columbiana* and *S. granulosa* as described in detail by Cook (1899: 675). The crests of the collum are very weakly developed, especially the median pair. Terminal segment trilobed, the notches only shallowly indented.

Male gonopods very complex, the terminal projections numerous and arranged as shown in the illustration. A seemingly characteristic flange-like development on the anterior side, this terminating in a slender spine, evenly curved laterad and caudad.

Dorsal color nearly uniform pale brown with a light median dorsal line, sides slightly lighter. Collum entirely white, in striking contrast with the rest of body. Underparts colorless to whitish-gray.

Relationships.—So little is known about the other eastern members of this genus (*granulosa* Bollman and *columbiana* Cook) that it is at present impossible to say anything definite about affinities. A careful study of good series of Striariae, including especially microscopic preparations of male genitalia, would probably reveal that the genus is excellent for zoogeographic and evolutionary investigations.

Ecology.—This species is definitely to be associated with hardwood forests on shady, well-drained hillsides. One can find numerous specimens by carefully lifting up the larger leaves and bits of bark in the upper humus layer. All of the specimens of this species (and others collected by me) have been found curled up. When placed in the hand or on a similar flat object the millipeds move about very awkwardly, usually waving the anterior third of the body about in the air. The type series of *zygoleuca* was taken in association with numerous immature specimens of the glomerid *Onomeris australora.*

The name *zygoleuca* refers to the white collum.

[1] This and the following ordinal names are those adopted for use in the forthcoming Checklist of North American Diplopoda.

North Carolina. Macon County: Satulah Mountain at Highlands, 4100 feet, July 20–24, 1949.

Tennessee. Sevier County: Chimneys Camp area, Rt. 71 between Newfound Gap and Gatlinburg, July 28, 1949 (two females provisionally placed here on the basis of color pattern.)

Family *Lysiopetalidae*

Spirostrephon highlandensis, new species

Plate 5, figs. 5, 6

Type locality.—Highlands, Macon County, North Carolina.

Type specimens.—Holotype and female allotype, U. S. Nat. Mus. no. 1874; several male and female topoparatypes in my personal collection and one deposited in the Highlands Museum, collected July 10–11, 1949.

Diagnosis.—Size very large, exceeding other known American lysiopetaloids, differing from *S. magnum* in having the usual six primary crests on posterior segments and from *lactarium* in much greater size and in the male gonopods.

Description of male holotype.—Length, 52.5 mm.; greatest width, 3.1 mm.; number of segments 58.

Eyes composed of 56 ocelli on the right side, 57 on the left, in 10 rows counting downward from the anterior corner, disposed as follows: 2, 3(4), 4, 5, 6, 7, 8, 8, 7, 6. Sense organ adjacent to the 5th and 6th rows of ocelli.

Antennae slender and rather short, not exceeding posterior edge of the fifth segment. Relative lengths of articles, from longest down, as follows: 3, 2, 5, 4, 6, 7, 1. The sixth is considerably swollen.

Collum with 20 even-sized crests.

Transition to full number of dorsal crests appears to take place on segment 10.

Two enlarged pleural crests below poriferous carinae, the tips of these bent slightly dorsad.

Primary and secondary crests differentiated over most of body. On segments posterior to 10th, there are three primary, four secondary, and four tertiary crests on each side of the mid-dorsal line.

Male gonopods large, black. Coxal process large, with a serrate angulation at midlength; distally truncate and bent laterad. Tibiotarsal portion broadly rounded distally. Branched process at base of tibiotarsus with two primary arms, nearly equal in size and length. The upper bears a small slender dorsal spine about one-third its length from the base; it is evenly tapered distad and slightly exceeds the lower arm. Latter evenly tapered to end, where expanded into a terminal cup-like development.

Color uniform dark brown dorsally with a yellowish narrow median stripe. Underparts and legs yellowish tan. Front of head light brown. Poriferous keels yellowish.

Description of female allotype.—Length, 45 mm.; width, 3.6 mm.; number of segments, 53.

Eyes composed of 55 ocelli on each side, in ten rows.

Agreeing with male in most other respects as regards structural characters. Color slightly lighter brown, with the middorsal light line broader than in the male.

Variation.—Of six type specimens at hand, the size ranges from 37.5 to 64 mm., both extremes represented by females. The average length for the series is 50 mm. The female 64 mm. in length appears to be the largest specimen known for the genus and the entire family as represented in North America.

The number of segments varies from 53 to 59, the two males having 57 and 58, the four females 53, 54, 58, 59. There seems to be no correlation between size and segment count, the female of 64 mm. having 59 segments, that of 37 mm. having 54.

Ocellus count varies slightly, the total number of 56 appearing to be the average. The ocelli are arranged in 10 rows, the median six of which (4th through 9th) are constant in number of ocelli. In one case there is an extra single ocellus at the front corner, actually making a count of 11 rows. The tenth row may have either 5 or 6 ocelli.

Relationships.—In size of the animals and in the configuration of the male genitalia, this species is very close to *S. magnum* Loomis from northern Alabama. That form, however, is very distinct in the presence of eight primary crests on the posterior tergites. The gonopods of the two species are very similar, but in *magnum* the end of the coxal process is recurved caudad and is drawn out instead of being truncate.

Northward through the Appalachians occurs another very large *Spirostrephon* the description of which is in preparation. This species, of which material has been seen from North Carolina, Virginia, West Virginia, and New York inhabits the mountains to the exclusion of the smaller, lowland *lactarium*. It is very similar to *magnum* and *highlandensis*.

Ecology.—A decided preference for oak-hickory climax forest is noted for this species. It inhabits well-drained situations, and frequently occurs under stones in dry places, a trait also noticed in *S. lactarium* in central Virginia. There is a tendency for the animals to congregate, and usually several can be found at once.

Highlandensis, for the type locality.

North Carolina. Macon County: Highlands, 4000 feet, July 10, 1949. Dry Falls, 2 miles west of Highlands, 3400 feet, July 19, 1949. Wesser Bald, Nantahala Mountains, near Kyle, July 29, 1949.
Transylvania County: Rt. 64, 2 miles west of Lake Toxaway, July 15, 1949.
Tennessee. Sevier County: Chimneys Camp, Rt. 71 between Newfound Gap and Gatlinburg, July 28, 1949.

Delophon carolinum, new species

Plate 5, fig. 4

Type locality.—Satulah Mountain, Highlands, Macon County, North Carolina, at 4100 feet.

Type specimens.—Holotype and female allotype in the U. S. Nat. Mus., no.

1875; a topoparatype female in my personal collection and one in the Highlands Museum; July 20–24, 1949.

Diagnosis.—Similar to *D. georgianum* Chamberlin (1943: 14) but with fewer ocelli and differences in the male gonopods.

Description.—Width of male type, 1.2 mm., of allotype, 1.6 mm.; both 25 mm. in length.

Ocelli in 9 rows as in *georgianum* but the total number much reduced. Ocellus count in the type: 1, 2, 3, 4, 5, 6, 7, 8, 9 = 45 on the right side, and 2, 3, 4, 5, 6, 7, 8, 8 = 43 on the left.

Generally similar in structural characters to *Spirostrephon* but the poriferous keels are inconspicuous and not pyriform or swollen as in that genus.

Color blackish with a narrow middorsal line.

Male gonopods with the lower (or cephalic) terminal branch longer and larger than the other, a reversal of the condition in *georgianum*.

Relationships.—Close to *georgianum*, the only previously known member of the genus.

Ecology.—This species appears to be somewhat scarce at Highlands, where only a few were found despite searches which revealed large numbers of other species. It has been found there only in the oak-hickory woods on Satulah Mountain, in dry leaves and beneath pieces of bark. At Reed Creek Falls two specimens were found in the humus in a rhododendron thicket but on a well-drained slope.

North Carolina. Macon County: Satulah Mountain, Highlands, 4100 feet, July 20–24, 1949. Georgia. Rabun County: Reed Creek Falls on Glade Mountain, July 27, 1949.

Family *Cleidogonidae*

Cleidogona jocassee, new species

Plate 7, figs. 15–18

Type locality.—Jocassee, Oconee County, South Carolina.

Type specimens.—Holotype and female allotype, U. S. Nat. Mus. no. 1876; ten topoparatypes in my personal collection, one in the Highlands Museum; all collected July 14, 1949.

Diagnosis.—A member of the *major* group of *Cleidogona, i.e.*, with the length 20 mm. or more; anterior division of the male gonopods straight and distally bifid; posterior division upright, simple, clavate. Distinct in the configuration of the gonopods and ninth male legs as illustrated.

Description of male holotype.—Length, 21 mm.; width at 7th segment, 2.4 mm.

Ocelli in a triangular patch, in 7 rows counting downward, from the front corner as follows: 1, 2, 3, 4, 5, 6, 6 = 27.

Configuration of body agreeing in all respects with the description given by Cook and Collins (1895: 42).

Color dorsally generally light brown or tan. Caudal margin of each tergite colorless. A small rounded whitish spot on each side of middorsal impressed

line, and a larger, oblong blotch on each side of the metatergites. Exposed portion of protergites darker brown along midline. Pleurites yellowish white. Legs uniformly white except terminal two joints, which are purplish. Front of head dark brown between the eyes, vertex marked with numerous fine parallel light lines. Three rounded light spots between the antennae. Clypeal region yellowish-white.

Gonopods large and prominent. Anterior division stout, nearly straight, distally bifid with the upper portion larger, somewhat expanded, finely setose; lower or anterior portion smaller, forming a thin, mesially directed lamina as seen in cephalic aspect. Posterior branch basally slender, straight, becoming expanded distally, notched just below the cephalic corner.

Ninth legs five-jointed, first and second subequal in length, the first globosely swollen basally, angularly produced distally; second slender over basal half, parallel, becoming swollen distally and abruptly contracted for insertion of tarsal joints. Latter subequal in size, third slightly the longest, the three combined shorter than half the length of the basal joint of the leg. The sternite is acutely produced upward laterad of insertion of leg.

Description of female allotype.—Length 22.4 mm.; width, 2.5 mm. Agreeing with male in structural features and in color, except the difference in body proportions.

Gonopods with the basal "receptacle" produced into two high, nearly straight, parallel-sided lobes as illustrated (the shape of these varying considerably with individuals). Valves quadrate in distal aspect, slightly larger cephalad, the upper surface strongly denticulate.

Relationships.—The closest relative of this form appears to be *Cleidogona major* Cook and Collins (1895: 47), known from Virginia and Maryland. *C. jocassee* is somewhat smaller and differs in gonopod characters, among which may be mentioned the much less swollen distal portion of the posterior branch and absence of the pronounced angular shoulder on the caudal margin of the anterior division. The terminal modification of the latter is also quite different. The sternite of the 9th pair of male legs is also acutely produced upward in *jocassee* and the second joint is much shorter in *major*. These differences, while significant, are only matters of degree, and the possibility that the two species intergrade cannot be precluded. Until such a time as extensive collections can be made in the Piedmont of North Carolina, however, I think it best to use full specific status for the present form.

Ecology.—This species was fairly abundant at the type locality, where it has a wide range of habitat types. Most of the Jocassee specimens were found in the leaf mold in a deep, shaded, cool ravine, very moist, with the forest cover chiefly yellow poplar, maple, hickory, and sweet gum. Undergrowth was chiefly rhododendron and laurel, and the ground was covered in many places with the interesting plant *Shortia galacifolia*. The pH of the humus was approximately 4.5 to 5. Other diplopods were scarce in the area, only *Cherokia georgiana* occurring in the same habitat. A few small scolopendrid and lithobid centipeds were observed. Specimens of the salamander *Plethodon clemsonae* were taken from the region as above characterized.

C. jocassee was later discovered to be common in the same sort of habitat elsewhere. *C. major*, at least where I have collected it in Piedmont Virginia, prefers hardwood forests and has not yet been found in ericaceous thickets.

North Carolina. Macon County: Highlands, chiefly Satulah Mountain, 4100 feet, July 9–30, 1949.
South Carolina. Oconee County: Jocassee, July 14, 1949.
Georgia. Rabun County: Reed Creek Falls on Glade Mountain, July 27, 1949.

Cleidogona margarita, new species

Plate 7, figs. 19–22

Type locality.—Chimneys Camp Area, Great Smoky Mountains National Park, Sevier County, Tennessee, on U. S. Rt. 71 between Newfound Gap and Gatlinburg.

Type specimens.—Holotype and female allotype, U. S. Nat. Mus. no. 1877; three topoparatypes also in the National Museum and one in the Park Headquarters collection at Gatlinburg, Tenn.

Diagnosis.—Distinct from all other known species as follows: ninth leg of males with a long, slender, clavate process from the distal end of the ventral face of the coxo-femur; receptacle of female genitalia, as seen in caudal aspect, produced into two upright lobes on each side as figured.

Description of male holotype.—Length, 18 mm.; width at 7th segment, 1.7 mm.

Ocelli in a triangular patch, arranged in 7 rows perpendicular to median line of the head, as follows: 1, 2, 3, 4, 5, 6, 7 = 28.

Configuration of body agreeing in all particulars with the description of the genus by Cook and Collins (1895: 42).

Color dorsally light brown, each segment with a broad dark gray band, this enclosing an oblong light spot on each side (extending as low as the lateral setigerous tubercule) and a small white dot near the median line, as usual in the genus. Sides and legs pale yellowish-white except tarsi which are brown. Sides of head and lateral light blotches finely reticulated with brown.

Gonopods of the *major* type, upright, nearly straight, slender, the anterior division distally bifid, the lower branch small, acuminate, the upper larger, somewhat flattened. Caudal margin of anterior gonopods as seen in lateral aspect abruptly produced caudad, forming an angular shoulder. Cephalic margin evenly lobed just below midlength. Posterior division simple, slender, distally clavate.

Ninth legs five-jointed, first slightly shorter than second, acutely lobed at base, distally produced into a long, slender, clavate branch. Second joint slightly bowed, thickest near midlength as illustrated. Basal two tarsi equal in size, expanded distally producing a somewhat moniliform aspect, terminal tarsal joint longer than basal two, slightly bacilliform, constricted beyond middle.

Other legs normal for the genus.

Description of female allotype.—Length, 17.5 mm.; width, 2 mm. Agreeing with male in most structural characters and in color.

Gynopods with the basal receptacle produced into four upright distinct lobes as illustrated. Valves quadrate in distal aspect, the upper surfaces conspicuously denticulate.

Relationships.—Although the male gonopods suggest affinity with *C. major* and *C. jocassee*, the nature of the ninth male legs and of the female genitalia precludes any close association of *margarita* with any presently known species. When our knowledge of the cleidogonid fauna is more complete, it may become desirable to set this species off in a separate genus.

Cook and Collins (1895: 21) mention "an aperture near the distal end of the second joint of the ninth legs of Cleidogona." I have not observed this in any of the available material but there appears to be an opening at the base of the first joint.

Ecology.—The type series was collected on a shady, north-facing hillside, with the dominant forest cover tulip poplar, magnolia, maple, and some hemlock. Undergrowth chiefly ericaceous shrubs. The millipeds were found under slabs of bark and in moist leaf mold, particularly around the bases of ferns whose dead fronds formed a sort of tent-like shelter.

Named for Mrs. Margaret Cannon Howell, in recognition of her interest in biological research at Highlands and in appreciation of many kindnesses.

Distribution.—Known only from the type locality.

Cleidogona inexpectata, new species

Plate 7, figs. 23–25

Type locality.—Chimneys Camp Area, Great Smoky Mountains National Park, Sevier County, Tennessee, on Rt. 71, between Newfound Gap and Gatlinburg.

Type specimens.—Male holotype and topoparatype, U. S. Nat. Mus., no. 1878; collected on July 28, 1949.

Diagnosis.—Similar to *jocassee* but with obvious differences in the male gonopods and ninth legs.

Description.—Length of holotype, 19.5 mm.; width, 1.6 mm. The structural characters are in all respects typical of other members of the genus. Ocelli as in *jocassee*.

Anterior division of male gonopods rather stout, distally bifid with the cephalic portion forming a long slender mesially directed lamina, caudal portion slightly larger, finely setose. Caudal margin of anterior gonopods with a prominent knob just above midlength. Posterior gonopods simple, distally clavate.

Ninth legs of male with first joint bearing a raised circular projection at its base, also a rounded knob distally. Second joint much less swollen than usual for the genus.

Relationships.—Judged from the genitalia, the closest known relative of this species would be *C. major*.

Ecology.—Taken in association with *margarita* (*q.v.*).

Inexpectata, unsuspected, surprising. The two types were assumed to be *margarita*, of which a long series was obtained, and were recognized as distinct only by accident.

Distribution.—Known only from the type locality.

Order POLYDESMIDA

Family *Xystodesmidae*

Boraria carolina (Chamberlin). Bull. Univ. Utah **30**(2): 6, 1939.

Plate 6, fig. 14

Specimens representing this species were obtained at a number of localities, extending the known range widely. Previously reported from Soco Gap Falls, N. C., by Chamberlin (*op. cit.*) and from Mount Rogers, Virginia, by Hoffman (1949: 379); the distribution may now be said to coincide with the Southern Appalachian mountains from Grayson County, Virginia, south to Rabun County, Georgia. It is known from the Blue Ridge, and from the Iron, Balsam, and Nantahala ranges.

A species described as *Aporiaria fumans* by Prof. Chamberlin (1943: 37) from the Great Smokies may well be identical with *carolina*. The male gonopods are quite similar, the color patterns identical, and size about the same.

The dark, nearly black, color of the pleurites and anal valves immediately distinguish *Boraria* from all other genera of the *Xystodesmidae* known from eastern United States. Further generic characters are the short femoral spines, rather small, high keels, and small but definite tubercules on the posterior metatergites.

The species appears to be somewhat scarce on the Highlands Plateau, where I found only two specimens. Perhaps it prefers somewhat lower elevations, for it is abundant along the Chattooga River in southeastern Jackson County, N. C., where I obtained a number of specimens of both sexes under boards and in heaped up flood debris. The Highlands specimens were both taken in rather wet hemlock-rhododendron woods near small streams.

North Carolina. Macon County: Highlands, July 13, August 1, 1949. Wesser Bald, Nantahala Mountains, near Kyle, July 29, 1949.
Jackson County: Chattooga River near Grimshawes P. O., July 10, July 24, 1949.
Georgia. Rabun County: Glade Mountain at Reed Creek Falls, July 27, 1949.

Cherokia georgiana (Bollman). Proc. U. S. Nat. Mus. **11:** 344, 1888.

Plate 5, figs. 9–12

Fontaria georgiana Bollman, op. cit.

Fontaria tallulah Bollman, op. cit., p. 345.

Mimuloria ducilla Chamberlin, Bull. Univ. Utah **30**(2): 7, 1939.

Mimuloria furcifer Chamberlin, Ent. News **51:** 282–83, 1940.

Mimuloria georgiana Loomis, Bull. Mus. Comp. Zool. **92**(7): 402; Loomis, Psyche **51:** 173, 1944; Loomis and Hoffman, Proc. Biol. Soc. Wash. **61:** 52, 1948.

Dynoria parvior Chamberlin, Proc. Biol. Soc. Wash. **60:** 10, 1947.

Cherokia georgiana Chamberlin, Proc. Biol. Soc. Wash. **62:** 3, 1949.

This widespread species is probably the most abundant xystodesmid in the Southern Appalachians, and is represented by many specimens from almost

every station at which I collected. The material at hand shows considerable variation in characters, and some attention may profitably be paid to this subject.

Under the generic names *Mimuloria* and *Dynoria*, several species have been recently proposed in this genus by Prof. Chamberlin (1939, 1940, 1947). The first of these, *Mimuloria ducilla*, has previously been synonymized with *georgiana* by Loomis (1943: 402) who had examined Bollman's original types in the National Museum. *M. furcifer* was described from Asheville, N. C., and established on the basis of larger size, somewhat different coloration, and presence of a small denticulation on one side of the tibiotarsus of the male gonopod. The latest name, *parvior*, described as a species of *Dynoria* and contrasted with the type of that genus (*icana*), appears not to be different from *georgiana*.

Judged from available literature records and some museum specimens, *Cherokia* as a genus is well distributed, having been reported from west Florida, Alabama, Georgia, Tennessee, and North Carolina. It is now possible to add South Carolina. Apparently the northernmost locality for the genus is Asheville, N. C., and it is possible that the French Broad River forms a barrier of some effectivity to further northward dispersal.

In the 25 male specimens on hand from six areas in the southern Appalachians, I find a complete gradient in all of the diagnostic characters heretofore appealed to.

The gonopods are strikingly similar in all of the material. The presence or absence of an angulation on the lateral side of the tibiotarsus, utilized as a character of *furcifer*, cannot be relied upon, as the following tabulation will indicate.

Locality	*Present*	*Absent*
Western S. C. and northern Georgia	2	1
Highlands Plateau	4	5
Nantahala Mountains	3	—
Soco Gap Falls	1	6
Smoky Mountains	1	2

In the Highlands material there is complete transition in this character, and in several cases it is difficult to say whether or not an angle is present. The extremes in this series are shown on Plate 5, figures 9 and 10.

47 adult specimens measured show a variation in size from 6 to nearly 9 mm. in width. There is no correlation between locality and size range. Loomis and Hoffman (*op. cit.*) have previously noted the considerable width variation in this species.

Color pattern affords the best geographic variable, but even this breaks down in large series. The Soco Gap population, described as *ducilla*, generally has the median spots lunate and sometimes in contact with the lateral series, but this is found chiefly in females and some of the males have very small median dots. At Highlands there is a notable tendency for adults to have a broad orange band across the caudal margin of the tergites, this anomaly occurring in 9 of the 18 specimens before me.

In view of the preceding evaluation of characters, I cannot conclude other than that *georgiana* is a highly variable, wide-spread species in which incipient raciation is evident; and that *Cherokia* at present must be regarded as a monotypic genus.

The ecology of *georgiana*, as well as its distribution, points to the fact that it is an aggressive, highly successful, and probably fairly recent form. Occurring in some numbers at nearly every station visited, it is euryzonal as regards habitat, and has been found in evergreen stands, in oak-hickory forest, and in dense rhododendron thickets—in short, everywhere I have found millipeds in the area.

Large numbers of immature specimens are to be found during the summer months, the majority of those observed in the last week of July being in the penultimate instar and having the adult color fairly well developed. *Fontaria tallulah* Bollman (*op. cit.*) was based on one of these forms, in which the keels are frequently red instead of yellow. No specimens have been found in copulation, this apparently taking place in a definite spring mating season as opposed to the summer-long period of certain other millipeds.

A female was dug up in deep leaf mold on July 23 at Highlands apparently just after she had commenced to deposit eggs. About 50–60 were in the cluster. These doubtless hatch before winter and the young mature the following summer.

An interesting situation has been observed in connection with the female genitalia. In these the outer valve is flattened and much larger than the inner, and no trace of a basal element (the receptacle) can be found. The latter is much reduced in certain genera (*e.g.*, *Nannaria*) but has not heretofore been found wanting. The disparity in sizes of the valves suggests relationships with *Zinaria*, as does the nature of the sternites and male gonopods. The genitalia are illustrated.

North Carolina. Macon County: Highlands, 3900–4200 feet, July 9–31, 1949. Wesser Bald, Nantahala Mountains, near Kyle, 2500 feet, July 29, 1949.
Jackson County: Chattooga River at Grimshawes P. O., July 10, 1949. Soco Gap Falls, Balsam Mountains, between Cherokee and Waynesville on Rt. 23, July 28, 1949 (topotypes of *ducilla*).
South Carolina. Oconee County: Jocassee, July 15, 1949.
Pickens County: Sassafras Mountain, Rt. 178, 15 miles NW of Pickens, July 15, 1949.
Georgia. Rabun County: Glade Mountain, about 5–7 miles south of Highlands, July 27, 1949.
Habersham County: Tallulah Gorge, July 18, 1949 (topotypes of *tallulah*).
Tennessee. Sevier County: Great Smoky Mountains National Park, near Chimneys, July 28, 1949.

Howellaria, new genus

Generotype.—*Aporiaria deturkiana* Causey (1942: 169).

Diagnosis.—Composed of head and 20 segments. Pore formula normal, pores opening dorsally on posterior third of keels. Latter directed conspicuously ventrad, dorsum highly arched. Keels with a notch on the caudal margin at point of insertion on tergite (suggesting the condition found in some cyclodesmids).

Tergites smooth and shining. Sternites flat, median constriction not obvious, caudal margin not lobed or spined. Prefemora caudad of gonopods with large, slightly curved spines. Femora spined as usual in the family. Male gonopods similar to those of *Boraria* (type, *carolina*) but the numerous other structural differences preclude any close association of the two genera. *Howellaria deturkiana* differs trenchantly from the forms of *Boraria* in (1) the highly arched, smooth dorsum (nearly flat and definitely wrinkled in *Boraria*), (2) the presence of large spines on the prefemora of the walking legs, (3) the color of the pleurites and anal valves being yellowish-tan instead of black, and (4) the terminal tarsal joint being conspicuously longer than the basal two, and much longer than femur. The general facies of the two genera are decidedly different. Plate 6 illustrates the differences in the 12th segment.

A further discussion of the relationships of the genus is planned for inclusion in a forthcoming account of the Xystodesmidae.

It gives me a great deal of pleasure to be able to name this unusual genus for Prof. Thelma Howell, in recognition of her interest in promoting knowledge of the Highlands fauna, and in appreciation of many kindnesses which served to make my work at Highlands the more enjoyable.

Howellaria deturkiana (Causey). Ent. News **53:** 169, 1942.

Plate 5, figs. 7, 8; plate 6, fig. 13

A single male topotype was obtained by digging in wet humus in a rhododendron thicket at Highlands. It agrees in every respect with Causey's description. The gonopods are figured herewith in two aspects.

North Carolina. Macon County: Highlands, edge of Lake Ravenel, July 13, 1949.

Nannaria austricola, new species

Plate 8, figs. 26, 27

Type locality.—Satulah Mountain, Highlands, Macon County, North Carolina.

Type specimen.—Adult female holotype, U. S. Nat. Mus. no. 1879, collected on July 26, 1949.

Diagnosis.—Immediately distinguished from all known species of *Nannaria* by the enormous spines of the femora of the walking legs. These are much longer than the femur itself and nearly the length of the tibia. The gynopods are probably also of specific diagnostic value.

Description.—A rather small species. Length of type, 22 mm.; width, 4 mm.

Structural features in general as described for various other members of the genus (e.g. *morrisoni* Hoffman, 1948: 348).

Color dark olive-gray as usual in the genus, edges of collum and caudolateral halves of keels pink. Underparts white.

Typical ambulatory leg from midbody as illustrated.

Gynopods with valves subequal in size, elongate, larger and proximally lobed

toward the attached end (insertion of oviduct), the upper edges of the valves shallowly scalloped; inner portions of valves excavate, forming a concavity with the suture along its middle. Receptacle much reduced, consisting merely of a small, subtriangular plate, loosely connected to the valves. Gonopodal aperture without special raised marginal flanges.

Relationships.—Since all previous workers have without exception ignored the taxonomic characters afforded by female genitalia it is at present very difficult to predict just where the affinities of this species lie. As far as known forms go, *N. morrisoni* of the Virginia Blue Ridge has the femoral spines approaching the size represented in *austricola* and the two species are about the same overall size.

In the material of *Nannaria* available to me, I find that the female genitalia provide excellent diagnostic features, and should by all means be utilized by milliped workers. A study of *Nannaria* is in preparation, in connection with which material of 17 species has been studied.

Ecology.—The single specimen was found in loose leaves in a dense, wet rhododendron thicket edging a small swift stream. This is precisely the conditions under which I have found most of the Nannariae that I have collected. An extended search over the immediate vicinity, as well as in other similar areas, resulted in the discovery of numerous immature specimens but no additional adults.

The name *austricola* is given in recognition of the fact that this is the southernmost member of the genus. In the lowlands of the Southeast, *Nannaria* is replaced by the closely related groups *Epeloria* and *Dicellarius*.

Distribution.—Known only from the type locality.

Nannaria scutellaria Causey. Ent. News **53:** 168, 1942.

Three topotypes were obtained in the Smokies in the area described above under *Cleidogona margarita*. They agree in all respects with the original description.

Tennessee. Sevier County: Chimneys Camp Area, Rt. 71, between Newfound Gap and Gatlinburg, Great Smoky Mountains National Park, July 28, 1949.

Sigiria rubromarginata (Bollman). Proc. U. S. Nat. Mus. **10:** 622, 1887.

Fontaria rubromarginata Bollman, *op. cit.*

Sigiria scorpio Chamberlin, Bull. Univ. Utah **30**(2): 9, 1939.

I have long suspected that Chamberlin's name *scorpio* actually applied to Bollman's species, especially since the former has been reported (Chamberlin, 1940: 56) from Soco Gap, N. C., only about 10 miles from the type locality of *rubromarginata*; and since the descriptions of the two match very closely.

On August 3rd I collected at Balsam, N. C., and was fortunate in obtaining three topotypical specimens, the gonopods of which are identical with those figured for *scorpio*. The animals were found in a very thin layer of moist leaves on a loose dirt bank, adjacent to the stream draining south from Balsam. The forest cover was yellow poplar and maple.

It is now possible to record this species from three localities, cited below. The range suggests a general occupancy of the northern part of the Great Smokies and extension eastward therefrom on such satellites as the Balsams, and perhaps the Cowees.

North Carolina. Jackson County: Balsam (Bollman, *op. cit.*), also a male and two females, August 3, 1949. Soco Gap Falls, April 29, 1939 (Chamberlin, 1940: 56).
Madison County: between Hot Springs and Paint Rock, August 7, 1910, R. V. Chamberlin (types of *S. scorpio*).

Sigmoria nigrescens, new species

Plate 8, figs. 28–32

Type locality.—Sassafras Mountain, on U. S. Rt. 178, one mile west of the intersection with S. C. Rt. 288, Pickens County, South Carolina.

Type specimens.—Male type and female allotype, U. S. Nat. Mus. no. 1880; a male topoparatype in my personal collection; July 15, 1949.

Diagnosis.—Similar to *S. divergens* Chamberlin (1939: 8) in configuration of male gonopods, differing from that species and others in lacking all traces of red or yellow pigment. Dorsum uniform bluish-black, the edges of the tergites narrowly unpigmented.

Description of male holotype.—Length, 43 mm.; width, 10.5 mm.

Structure as usual for this genus. Tergites rather flattened, keels wide, continuing slope of dorsum; finely coriaceous; prozonites smooth. Repugnatorial pores dorsal in position, in an ellipsoid swelling. Caudolateral corners of keels posterior to those of fifth segment becoming increasingly produced.

Sternites nearly flat, not produced into lobes or spines at bases of legs. Latter relatively long and slender, the prefemora with small ventral spines; femora with acute ventrodistal spine as usual. Tibiae long and slender. Terminal tarsal joint nearly as long as basal two combined.

Sternites anterior to gonopods without special lobes or processes.

Gonopods in a large rounded-ovoid aperture, without raised edges. Femoral portion massive, densely setose, with a small hook-like lateral femoral process. Tibiotarsus heavy, sigmoidally curved in a cephalolateral direction, its median side not produced into a thin broad lobe as in *latior* and related species; distal portion becoming flattened and somewhat twisted as shown in the drawings.

Dorsal color uniform bluish-black, with a very thin margin to the metatergites colorless. Sternites yellowish-white, legs white, lightly reticulated with black. A thin black mark on the pleurites beneath the keels. Antennae brown. First pair of legs white.

Description of female allotype.—Length, 39 mm.; width, 10 mm. Similar in most respects to male, differing only in proportions of body, greater arch of dorsum, etc.

Gynopods large, their aperture behind second pair of legs without raised marginal flange. Receptacle broadly horse-shoe shaped, the lateral caudal projections triangular in lateral aspect as illustrated. Both valves with the median dorsal portion excavated, the surface with conspicuous cross-ridges. Caudal

portion of each valve produced into a conspicuous knob, these in contact mesially as seen in distal aspect.

Relationships.—Obviously the closest relative of this form is *Sigmoria divergens* Chamberlin, described from Landrum, Spartanburg County, S. C., a locality at the eastern edge of the Blue Ridge escarpment and about 35 miles northeast of the type locality of *nigrescens*. The gonopods of the two are similar, but seem to be much shorter and heavier in *nigrescens*, also the color pattern of the latter is unique for the genus and probably for the family as well. My original surmise that the specimens had only recently molted was not borne out, for they were kept alive long enough for any incipient red pigment to manifest itself in the adult pattern.

These two species, with possibly *S. mimetica* (Chamberlin, 1918: 29) from central Tennessee, form a distinct subgeneric group based on similarity of the male gonopods. It is also interesting to note that the three occupy more or less peripheral positions on the general range of the genus. A further investigation, including a study of the female genitalia, should be of much value. Unfortunately, published drawings of the known Sigmorias show the male gonopods in such a diversity of aspects that comparisons cannot be accurately made.

Regarding the use of male genitalia as specific characters, it is of interest to record here some remarks on the subject by two careful American workers (Cook and Collins, 1895: 31):

"Dissection is an absolute necessity in studying the genitalia of the smaller forms, and the greatest caution must be exercised for the different views of the same irregularly shaped genitalium will look like entirely different structures. . . . This method has the further advantage of allowing corresponding parts to be viewed at the same angle, so that fictitious differences are not multiplied. . . . Descriptions of genitalia are nearly worthless without diagrams to aid in their interpretation."

It is a matter of some regret that later American workers have never paid much attention to such reasonable admonition.

Ecology.—The type specimens were found in a deep, narrow, moist ravine on the side of Sassafras Mountain, with the dominant forest cover tulip poplar, hickory, maple, and a few oaks. Near the stream were many shrubs of rhododendron. The millipeds were found in leaf mold along a path, where the debris had been piled up by water.

The specific name refers to the black color.

Distribution.—Known only from the type locality.

Sigmoria, species incertis

A single female of this genus obtained in the Nantahalas of western Macon Co., N. C., seems to be undescribed, but may actually be Chamberlin's *S. stenogon* (1942: 5). Although an extended search was made in the vicinity, no additional specimens could be found, and I feel it unwise to propose a new name in the absence of males. The color pattern is one of the most attractive I have seen in millipeds, and a description of the color from life may be of interest.

"Prozonites and front margin of metazonites shining black, rear two-thirds of metazonite light pearl gray as is tip of anal segment and rear edge of collum; entire dorsal surface of keels and front edge of collum bright vermilion. Underparts whitish, legs becoming pink distally. Antennae brown." (Field notes.)

Chamberlin (*op. cit.*) says of *stenogon*: "The preserved specimens have the tergites anteriorly brown with the caudal half and the keels yellow . . . etc."

North Carolina. Macon County: Nantahala Mountains, west side of Wesser Bald on Otter Creek, near Kyle, July 29, 1949. One female in humus in rhododendron thicket near creek.

Family *Polydesmidae*

Dixidesmus branneri (Bollman). Proc. U. S. Nat. Mus. **10:** 620, 1887.

Polydesmus branneri Bollman, *op. cit.*

Polydesmus conlatus Chamberlin, Proc. Biol. Soc. Washington **56:** 36, 1943.

A very characteristic and abundant species of the Great Smokies and other adjacent mountains, *branneri* extends north into Virginia as far as the latitude of Charlottesville and Hot Springs. It is, in the northern part of its range, definitely a montane form and restricted to high or cool habitats. *Branneri*, or a form of it, apparently occurs south as far as northern Florida (Gainesville) and elsewhere along the Gulf Coast. Floridian material is much smaller than Appalachian, but the male gonopods are identical. Chamberlin's description of *conlatus* (*supra*) recorded it from southern Georgia at Thomasville.

During the course of my Highlands work I obtained only a few specimens, in the Smokies, and these add nothing to the range of the species which has previously been recorded from Newfound Gap.

Tennessee. Sevier County: Chimneys Camp on Rt. 71 between Newfound Gap and Gatlinburg, several males taken July 28, 1949, in leaves on a shady north-facing hillside.

Dixidesmus tallulanus Chamberlin. Bull. Univ. Utah **34**(6): 19, 1943.

Apparently the dominant and only large polydesmid species on the Highlands Plateau. Great numbers of immature specimens were found during July, chiefly in oak-hickory forest, but the only mature males were obtained on July 22 and August 2.

D. tallulanus has previously been recorded only from several stations in Rabun County, Georgia; the following records provide the second locality for the species and the first record for it in North Carolina.

North Carolina. Macon County: Highlands, generally distributed and observed during July, 1949; adult male taken on south side Satulah Mountain, August 2, 1949. Dry Falls, 3 miles west of Highlands on Rt. 64, July 22, 1949 (J. M. Valentine).

Scytonotus granulatus (Say). Jour. Acad. Nat. Sci. Philadelphia **2:** 107, 1821.

Large numbers of immature specimens found at Highlands. This species does not mature until October or November and it is extremely difficult to collect

adults in the summer. My identification is wholly tentative, the Highlands population may be *S. virginicus* or an undescribed species.

North Carolina. Macon County: Highlands, generally distributed and observed during July, 1949.

Order SPIROBOLIDA

Family *Spirobolidae*

Spirobolus dolleyi (Loomis). Bull. Mus. Comp. Zool. **92**(7): 398, 1943.

The animal at hand presents some interesting and perplexing problems. My identification above is only provisional, the specimen merely being more like *dolleyi* than like *marginatus* or *orophilus*. In the present state of our knowledge, I find it extremely difficult ever to be really sure about some of the Spiroboli. A thorough revision of the genus is much needed here, as with other groups.

S. dolleyi has been reported from Mississippi and Alabama by Loomis (*op. cit.*), and I have some material from southwest Virginia that fits the description perfectly. The eastern forms may eventually all turn out to be subspecies of *marginatus*. The ranges would appear to be vicarious.

My adult male was found crawling across a trail through a rhododendron thicket and brought in just before my departure. The scarcity of spirobolids at Highlands and elsewhere during 1949 is somewhat remarkable, considering the usual abundance of the creatures.

North Carolina. Macon County: Highlands, Rhododendron Trail, August 1, 1949 (R. E. Gordon).

Order JULIDA

Family *Paraiulidae*

Ptyoiulus, species incertis

This form was rather abundant but no mature specimens of either sex could be found. Since it could equally well be any of the three species known from eastern United States (*pennsylvanicus, coveanus, georgiensis*) I hesitate to make a tentative specific determination.

The millipeds were almost altogether found in well-drained deciduous woods, in and around Highlands.

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EXPLANATION OF PLATES

PLATE 5

Fig. 1, 2. *Onomeris australora*, n. sp. 1, nineteenth male legs, ventral view, 2, female genitalia, caudal view.

Fig. 3. *Striaria zygoleuca*, n. sp. Left gonopod of male, caudal view.

Fig. 4. *Delophon carolinum* n. sp. Right gonopod of male, *in situ*, lateral view.

Fig. 5, 6. *Spirostrephon highlandensis* n. sp. 5, male gonopod in lateral aspect. 6, left male gonopod, caudal view.

Fig. 7, 8. *Howellaria deturkiana* (Causey). 7, left male gonopod, cephalic view. 8, the same, mesial view.

Fig. 9–12. *Cherokia georgiana* (Bollman). 9, 10, cephalic views of the tibiotarsi of the

gonopods of two males from Highlands, showing extremes in variation of the subterminal tooth. 11, lateral aspect of female gynopod. 12, distal aspect of same.

PLATE 6

Fig. 13. *Howellaria deturkiana* (Causey). Lateral view of two midbody segments, legs not shown.

Fig. 14. *Boraria carolina* (Chamberlin). Lateral view of two midbody segments, legs not shown.

PLATE 7

Fig. 15–18. *Cleidogona jocassee* n. sp. 15, lateral view of male gonopods. 16, cephalic view of same. 17, ninth male leg and part of sternite. 18, female gynopods, caudal view.

Fig. 19–22. *Cleidogona margarita* n. sp. 19, lateral view of male gonopods. 20, cephalic view of same. 21, ninth male leg. 22, female gynopods, caudal view.

Fig. 23–25. *Cleidogona inexpectata* n. sp. 23, lateral view of male gonopods. 24, cephalic view of same. 25, ninth male leg.

PLATE 8

Fig. 26, 27. *Nannaria austricola*, n. sp. 26, leg from midbody segment to show greatly developed femoral spine. 27, lateral view of female gynopod.

Fig. 28–32. *Sigmoria nigrescens*, n. sp. 28, left male gonopod, cephalic view. 29, the same, mesial view. 30, the same, caudal view of tibiotarsus. 31, lateral view of gynopod. 32, distal view of gynopod.

PLATE 5

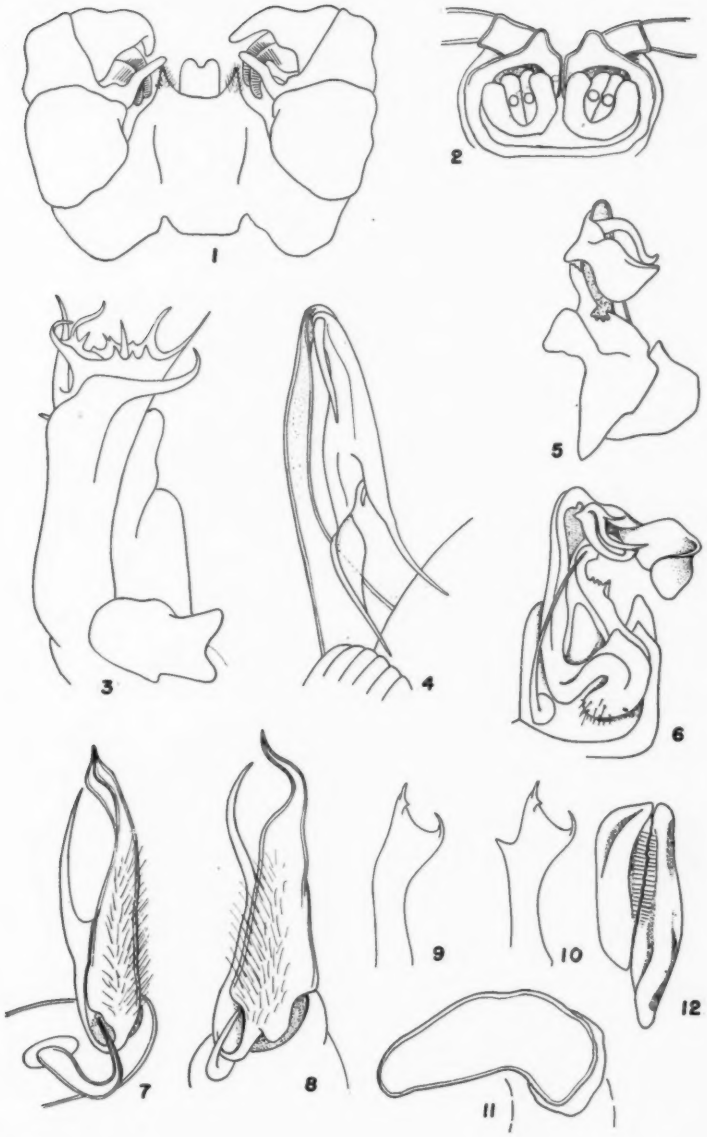
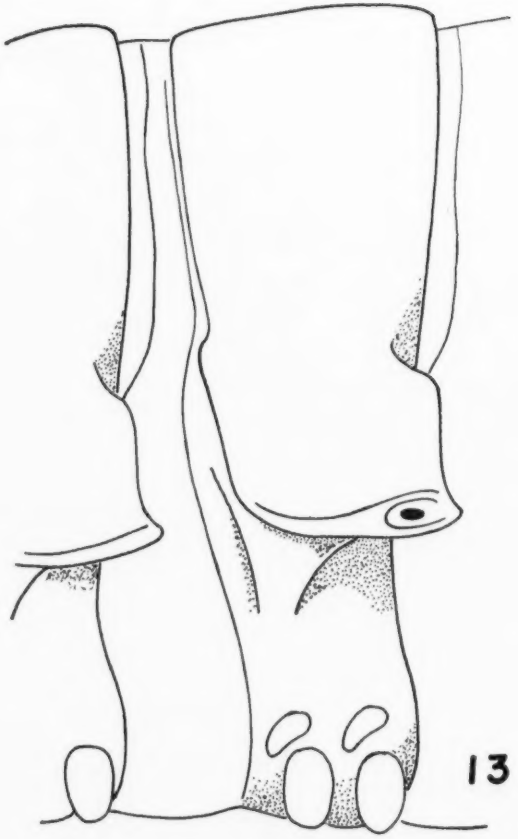


PLATE 6



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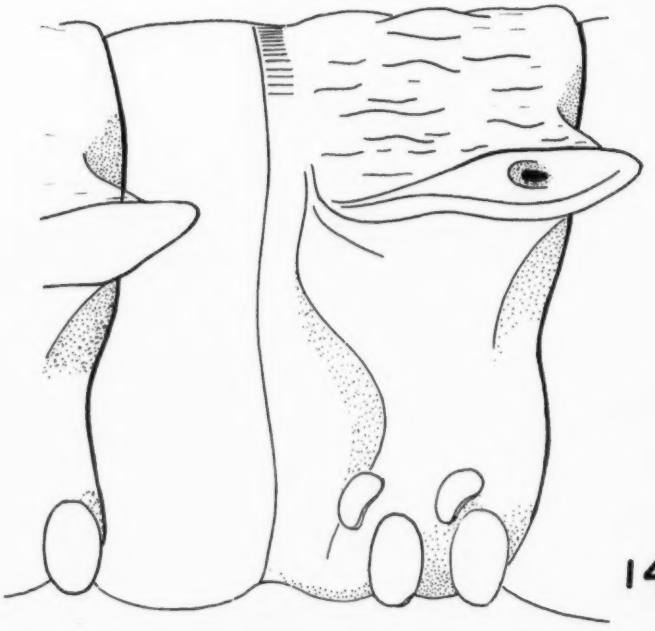

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PLATE 7

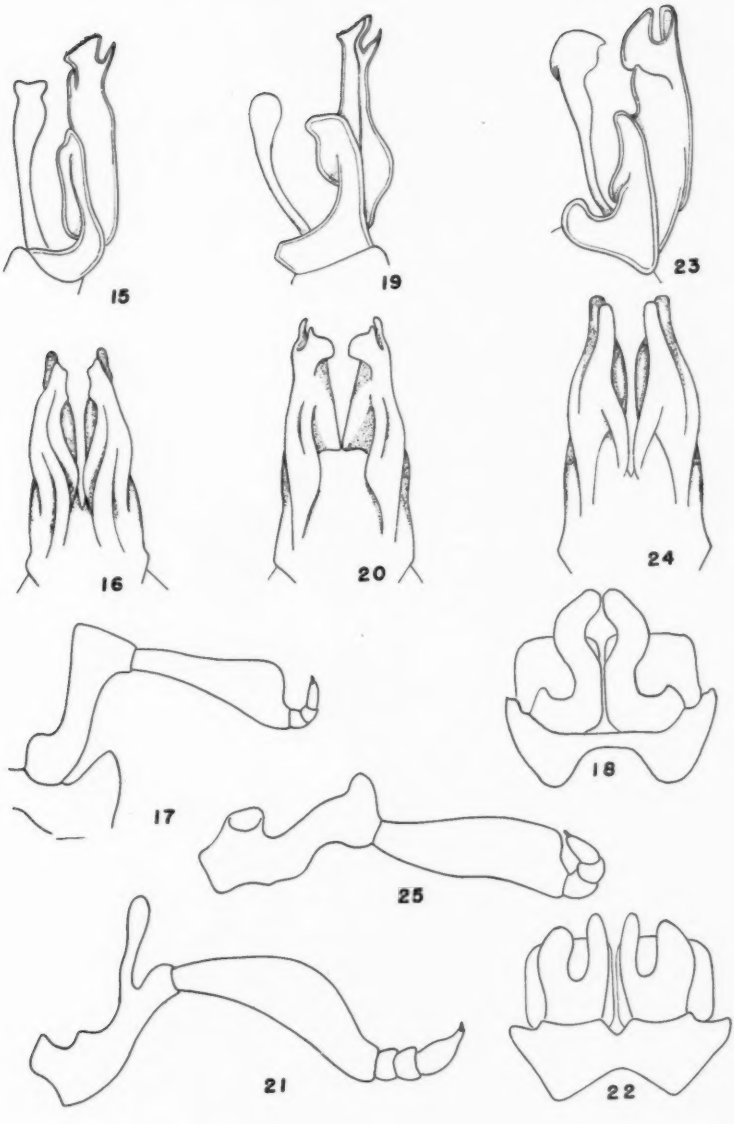
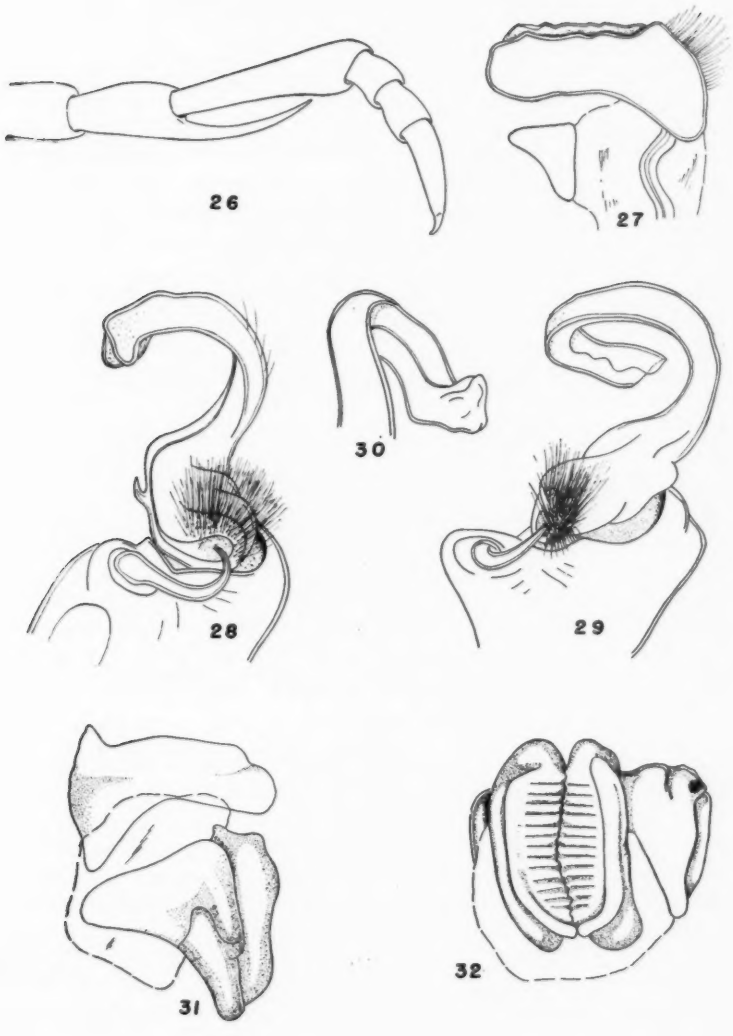


PLATE 8



STUDIES ON MYCOSPHAERELLA ROSIGENA[1]

By D. E. Ellis and C. N. Clayton

Plate 9

Mycosphaerella rosigena (E. & E.) Lindau was described on rose leaves from Louisiana (Ellis and Everhart, 1887; Saccardo, 1891). Although this fungus has been reported from a number of countries, comparatively little is known about its life history and pathogenicity. Available reports indicate that it is rather widely distributed on cultivated roses (*Rosa* spp.) and that at times it is associated with a leaf spot of considerable economic importance. In the United States the fungus has been reported from Louisiana, New Jersey (Halsted, 1894), New York (Stewart, 1910), Maryland (Weiss, 1942), Ohio[2], Tennessee[2], and North Carolina (Ellis and Clayton, 1948). It has been found in Porto Rico (Weiss, 1942), Panama (Weiss, 1942), Cuba (Bruner, 1921), Trinidad (Stevens, 1930), the Dominican Republic (Ciferri and Fragoso, 1926), Brazil (Averna-Sacca, 1926), Australia (Grieve, 1932), India (Sydow and Butler, 1911), Malta (Anon., 1913), and China (Ling, 1948). A variety, *M. rosigena* var. *madagascariensis*, was reported from Madagascar (Bouriquet, 1946). Also according to a letter from Freeman Weiss[2], there are references to its occurrence in Argentina, Bermuda, Venezuela, Ceylon, Italy, and Spain.

In the fall of 1947 *M. rosigena* was found consistently associated with a severe leaf spot defoliation of greenhouse roses (Better Times and Briarcliff varieties) in Raleigh, North Carolina. Brief descriptions of the symptoms of the disease and the cultural characteristics of the fungus were reported (Ellis and Clayton, 1948). Further observations on the physiology, morphology, and pathogenicity of the organism are given below.

SYMPTOMS

The symptoms observed in North Carolina agree in most respects with those given by Stewart (1910) who described the disease on greenhouse roses in New York. The grayish-brown leaf spot constitutes the most characteristic symptom (Pl. 9, fig. 1). A typical spot, is somewhat irregular in outline and has a necrotic center surrounded by a purple border. The spots range from about 0.5 to 5 mm. in diameter. The tiny perithecia of *M. rosigena* are borne in great numbers on necrotic areas of both leaf surfaces. Young spots not yet necrotic are purple in color and range in size from flecks just visible to the naked eye to about 3 mm. in diameter. Frequently, when these spots are very numerous, chlorosis develops in the areas between them and the leaflets abscise before they become necrotic.

[1] Contribution from the Plant Pathology Section, Department of Botany, North Carolina Agricultural Experiment Station, Raleigh, North Carolina. Published with the approval of the Director as Paper No. 338 of the Journal Series.

[2] Records on file with Division of Mycology and Disease Survey, Beltsville, Maryland, according to correspondence from Dr. Freeman Weiss.

The disease is apparently confined to the older, fully expanded leaves. Lesions occur abundantly on leaves below excised flower stalks, but none were observed on leaves of shoots that grew from axillary buds just below the most recently cut flower stalks. Severe defoliation sometimes resulting in a loss of 90 per cent of the foliage is associated with the disease.

PATHOGENICITY

M. rosigena was readily isolated from the very small non-necrotic (no visible necrosis) purple lesions as well as from large necrotic spots. In Table 1 are given the results of one series of isolations on potato-dextrose agar. Small pieces of affected tissue were cut from leaves, dipped in either a 1–1000 aqueous solution of mercuric chloride for 30 seconds or in a 0.5 per cent aqueous solution of sodium hypochlorite for 1 minute, washed in 4 changes of sterile distilled

TABLE 1

Number of colonies of M. rosigena *and other organisms isolated from rose leaflet lesions of various sizes*

DIAMETER OF LESIONS	SURFACE DISINFECTANT USED	NUMBER OF PLATINGS	NUMBER OF PLATINGS YIELDING	
			M. rosigena	Other fungi and bacteria
1 mm. or less (not necrotic	Mercuric chloride	24	17	2
	Chlorine	24	20	4
1 to 3 mm. (not necrotic)	Mercuric chloride	24	15	3
	Chlorine	24	20	18
3 to 12 mm. (necrotic)	Mercuric chloride	24	17	5
	Chlorine	24	22	14
Totals.....		144	111 (77%)	46

water, and transferred aseptically to potato-dextrose agar in Petri dishes which were kept at about 25°C. for 6 to 10 days.

The fact that *M. rosigena* was isolated about as consistently from both groups of small, purple, non-necrotic lesions as from the older necrotic spots bearing perithecia indicated strongly that the fungus was parasitic.

M. rosigena is generally considered to be pathogenic and has consistently been reported as occurring in necrotic spots on living leaves. However, no record of inoculation tests has been found.

Inoculations with pure cultures of the organism were made on young Better Times rose plants in a greenhouse at Raleigh, North Carolina, in 1948. Cultures about a month old were macerated in a Waring blender and atomized onto the leaves. The inoculated plants, together with non-inoculated control plants, were placed in a moist chamber for 48 hours and then removed to the greenhouse bench. About two months after inoculation sparse infection appeared on four of the eight plants inoculated. Perithecia with mature ascospores of *M.*

rosigena later developed on many of the affected leaves. The fungus was recovered in culture. No symptoms of the disease developed on four non-inoculated plants used as controls. In presenting the positive results of this inoculation test it is recognized that they are not conclusive and that additional work will be necessary to explain fully the pathogenesis of *M. rosigena*.

THE FUNGUS

Perithecia and asci of *M. rosigena* are shown in Plate 9, figs. 2 and 3. The fungus in North Carolina was found to conform in all important aspects to the original description (Ellis and Everhart, 1887) and to the drawings of Schwarze (1917). In comparing it with a portion from the type collection[3] and with *M. rosicola* B. H. Davis[4], it was found to conform to the former and differed from the latter as expected from the original descriptions of the two species (Ellis and Everhart, 1887; Davis, 1938).

Perithecia of *M. rosigena* occur abundantly on necrotic lesions on living leaves as well as on abscised dead leaves. Necrotic spots barely visible to the

TABLE 2

Relative abundance of perithecia of M. rosigena *on dorsal and ventral surfaces of necrotic lesions of various sizes*

LEAF SURFACE	NUMBER OF PERITHECIA PER SQ. MM. ON LEAF LESIONS OF STATED DIAMETERS		
	0–0.5 mm.	0.5–1.5 mm.	1.5–5.0 mm.
Dorsal	9	24	37
Ventral	11	17	27

naked eye may bear numerous perithecia on both leaf surfaces. Results of perithecial counts on dorsal and ventral surfaces of affected leaflets are shown in Table 2. The averages are based on perithecial counts in 20 lesions for each of the first two categories and 10 for the third one.

No record has been found of *M. rosigena* having been cultured. Experiments showed that it grew well on the following agar media which were prepared according to standard formulae excepting as indicated: potato-dextrose, potato-dextrose plus 0.1 per cent asparagin, bean, corn-meal, prune, rose leaf[5], rose leaf plus 2 per cent dextrose, and 2 per cent dextrose. After 33 days at 25°C. average colony diameters for 5 plates of each of the above media were 16.3, 15.3, 21.8, 13.5, 15.8, 35.5, 17.5, and 17.2 mm., respectively. These figures, however, do not indicate relative total growth as the colonies tended to be much more raised on some media than others. Preliminary studies indicate that the optimum temperature for growth in culture was between 20° and 30°C. Only a trace of growth occurred at 5°C. and none at 35°C.

[3] Loaned by Dr. John A. Stevenson.

[4] Loaned by Dr. L. M. Massey.

[5] 135 gms. dried rose leaves steeped in 500 ml. water at 90°C. for 1½ hours; decanted, 20 gms. agar added, and water added to bring volume to 1000 ml.

The colonies are olive-gray on potato-dextrose agar, have irregular margins, and form raised mounds of hard stroma-like tissue (Pl. 9, fig. 4) in which numerous perithecia are embedded. Immature perithecia were formed within 14 days and a few contained asci and ascospores after 21 days. Although immature perithecia were found within 26 days on all of the above named media except rose leaf agar, they had reached a mature condition only on potato-dextrose, potato-dextrose plus asparagin, and dextrose agars. They were most abundant on potato-dextrose agar. No asexual spores were observed.

SUMMARY

Mycosphaerella rosigena on cultivated roses is widely distributed in tropical and subtropical countries of the world and occurs in greenhouses in certain temperate regions of the United States.

The fungus was associated with a severe leaf spot of Better Times and Briarcliff roses growing in a greenhouse at Raleigh, North Carolina, in 1947.

The fungus was readily and consistently isolated from the tiny, purple, non-necrotic leaf spots.

In an inoculation test sparse infection and typical leaf spots containing perithecia and ascospores were obtained on 4 of 8 inoculated Better Times rose plants. Four control plants remained healthy.

M. rosigena was isolated in pure culture and grew well on several agar media. At about 25°C., on potato-dextrose agar, olive-gray raised colonies were formed in which numerous perithecia occurred after 14 days and mature ascospores were produced after 21 days.

Counts showed that perithecia occur abundantly in necrotic leaf spots on both leaf surfaces.

No asexual stage of the fungus was found in culture or on rose leaves.

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EXPLANATION OF PLATE 9

Mycosphaerella rosigena

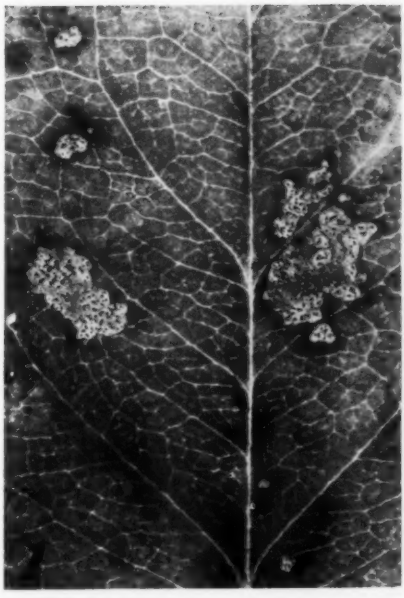
Fig. 1. Enlarged portion of Better Times rose leaflet with typical leaf spots containing numerous small perithecia. × 3.5 (approximately).

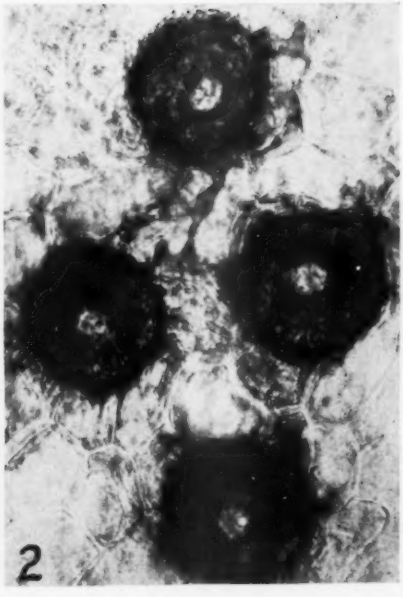
Fig. 2. Portion of cleared rose leaflet containing perithecia. × 350

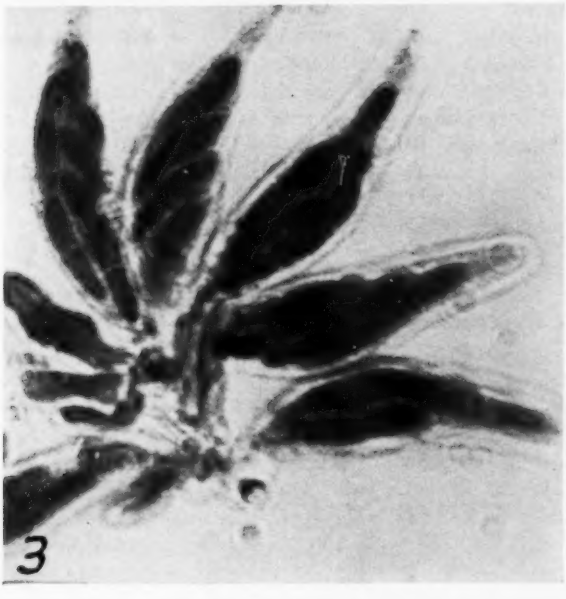
Fig. 3. Part of contents of a perithecium showing asci with ascospores stained with cotton blue. × 1666.

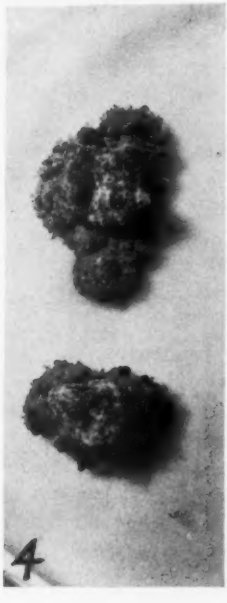
Fig. 4. Thirty-day-old colonies on potato-dextrose agar. × 1.25

PLATE 9









BOPYRID ISOPODS FROM THE COAST OF NORTH CAROLINA

By A. S. Pearse

Department of Zoology, Duke University
Durham, North Carolina

During the summer of 1949 collections were made from the reefs off the Carolina coast. Two species of parasitic isopods were found, one of which appears to be new. These and their hosts have been deposited in the United States National Museum.

Thanks are due to Dr. Louis G. Williams, who dived to depths of 12 to 50 feet. The United States Fish and Wildlife Service furnished working space at their Beaufort Laboratory, where Clinton Atkinson and Vance Fulford did everything possible to help the work.

Bopyro choprae Pearse

Figs. 1, 2

Two pairs were collected on August 8 from the Black Rocks six miles offshore at a depth of 45 feet, from the gill chambers of snapping shrimps, *Synalpheus longicarpus* Herrick and *S. minus* (Say). These agree with the descriptions in my 1932 paper, except that the terminal segments of the abdomens of the females (Figs. 1, 2) show some variation. This may be due to age or the size of the host, as the first host mentioned was much smaller than the other. The dwarf males were both attached to the abdomens of the females. They both have the terminal segment of their abdomen like the right hand picture in Figure 10 of the 1932 paper. This species was reported from Dry Tortugas, Florida, and from Bimini from a different species of alpheid shrimp, *Synalpheus brooksi* Coutière.

Diplophryxus synalphei n. sp.

Figs. 3–7

Female.—Deformed (Fig. 7); irregularly asymmetrical; the left side greatly swollen; form globular. No pigmentation. Eyes absent. Length 6.2 mm.

Head deeply sunk in the thorax; longer than wide; on dorsal side; surrounded posteriorly and laterally by first segment of thorax; anterior margin little curved compared to posterior margin. Antennae small, 1-segmented. Mouth parts not visible from dorsal side.

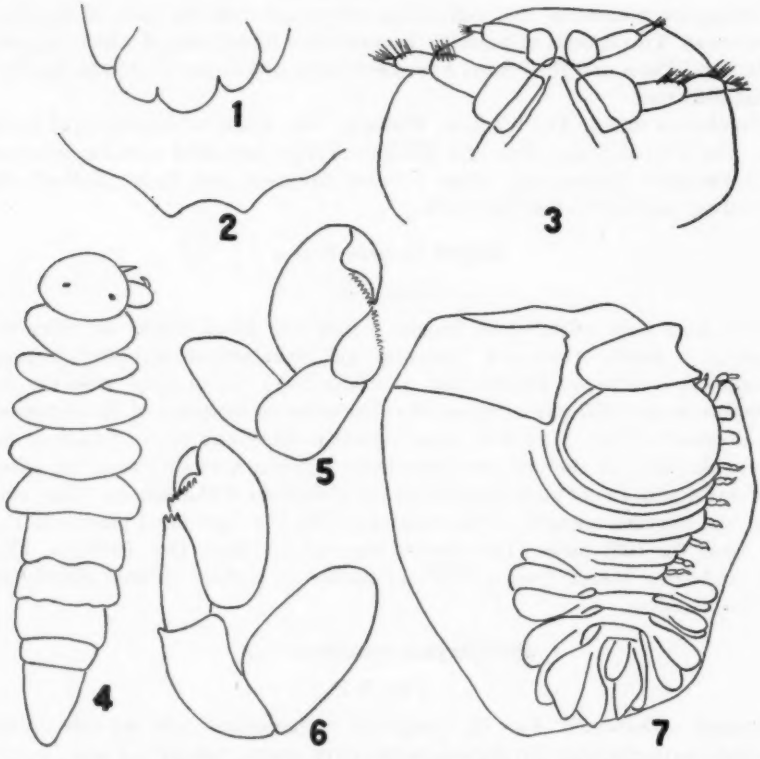
Seven thoracic segments gradually increase in length posteriorly; on the right side they are distinct and each bears a leg; on the left side only the first segment is defined and it only bears a leg. The legs are all small, and end in a small terminal claw.

The abdomen is 5-segmented; the first four segments bear biramous lamellate appendages; the last segment is rounded posteriorly and its lateral margins

are straight and nearly parallel; the appendages on the left side are slightly larger than those on the right.

Both females are ovigerous. Incubatory pouch large; lamellae on the left side are much larger than those on the right.

Male.—Both dwarf males (Fig. 4) were among the eggs in the incubatory pouch of the females. Depressed, slender; length 1.7 mm.



FIGS. 1–7. 1, 2. *Bopyro choprae*, terminal segments of female abdomen. 3–7, *Diplophryxus synalphei*: 3, ventral view of head of male; 4, dorsal view of male; 5, first right leg of male; 6, seventh left leg of male; 7, dorsal view of female.

Head wider than long; first and second antennae 3- and 4-segmented; small eyes present. Legs (Figs. 5, 6) stouter and with the propodus more spinulose on the margin contiguous to the dactyl than in the female.

Abdomen short (0.49 mm.), tapered toward the posterior end, rounded at tip, and rather feebly divided into three segments.

Occurrence.—Pairs were found on the abdomens of ovigerous female snapping shrimps, *Synalpheus fritzmulleri elongatus* Coutière from the Black Rocks off New River at a depth of 15 feet on July 7 and August 10.

Types.—U. S. Nat. Mus. Nos: Female, 90103; male, 90104.

This species differs from others in the genus Diplophryxus (Shino, 1934) in the shape of the last segment and of the head of the female and in the segmented abdomen of the male. Richardson's (1904) *D. jordani* has longer pedicles on the female abdominal appendages. Shino's (1934) *D. alphei* has some tri- and quadriramous pleopods on the female. Chopra's (1930) *D. richardsoni* and *D. kempi* differ in general form and in the shape and structure of the male and female appendages. Hay's (1917) *Phryxus subcaudalis* differs in the number and structure of the thoracic and abdominal appendages and of the last segment of the abdomen of the female; the male abdomen also is rounded distally and 3-segmented.

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CAROLINA BAYS IN RELATION TO THE NORTH CAROLINA COASTAL PLAIN[1]

By David G. Frey

Department of Zoology, University of North Carolina
Chapel Hill, North Carolina

The Atlantic Coastal Plain is a region of many problems and of widely conflicting opinions concerning a number of these. Two of direct importance in the present discussion are the questions of marine overlap onto the Coastal Plain with the possible production of "terraces", and the Carolina bays which occur on these terraces.

COASTAL PLAIN TERRACES

The idea of the Atlantic Coastal Plain being arranged in a series of broad steplike terraces, possibly representing successive sea levels higher than the present, was first conceived in Maryland. In 1907 Johnson described in general terms five of these terraces in North Carolina, and in 1912 Clark *et al.* published a map of the North Carolina Coastal Plain showing six terraces, of which the uppermost one, the Lafayette, was believed to be Pliocene in age and the other five Pleistocene. Fig. 1 shows a somewhat simplified arrangement of these terraces taken from this map; to avoid confusion the many reentrants of lower terraces along the larger streams have been omitted. The five Pleistocene terraces decrease in elevation towards the coast. The limits of these as given by Clark *et al.* are: Coharie 235–160 ft.; Sunderland 150–110 ft.; Wicomico 100–50 ft.; Chowan 50–25 ft.; Pamlico 25–0 ft. Cooke (1935) proposed that the Pamlico terrace was formed during the Peorian interglacial subage, the Talbot (= Chowan) and Wicomico during the Sangamon age, and the Sunderland and Coharie terraces during the Yarmouth interglacial age. No evidence is given for these datings other than the assumptions that the terraces were formed during interglacial ages when the melting of the glaciers made the sea level higher than at present, and that the terraces are arranged in chronological order from the youngest nearest the coast to the oldest at the inner border of the Coastal Plain.

Flint (1940) reviewed critically the evidence for terraces and rejected most of it as inadequate and too conflicting. On the basis of field studies he was able to find and trace only two wave-cut scarps in North Carolina, which probably represent eustatic changes in sea level. The lower one or Suffolk scarp follows approximately the boundary between the Pamlico and Chowan terraces as given by Clark *et al.*; its toe lies at an altitude of 20–30 ft. above sea level. The upper or

[1] This work is being carried out with the aid of a research grant in 1949, Project C-63 from the Carnegie Foundation for the Advancement of Teaching. Previous studies on Singletary Lake by the author referred to a number of times in this paper were assisted by a similar grant in 1948, Project C-50. The author wishes to acknowledge the assistance of Howard T. Odum in 1948 and Richard W. Borden in 1949 in performing the necessary field work and some of the laboratory work.

Surry scarp lies close to the inner boundary of the Wicomico terrace, although in some places it is 15 miles away from the boundary as shown by Clark *et al.* Its toe lies at an elevation of 90–100 ft. Flint states that there is no evidence in the Carolinas for any marine invasions higher than the Surry scarp. This, if generally accepted, would make untenable any theories regarding the Carolina bays as having arisen along the sea coast or in shallow marine locations, unless they are exceedingly old. Flint also states that the general concept of terraces in the sense

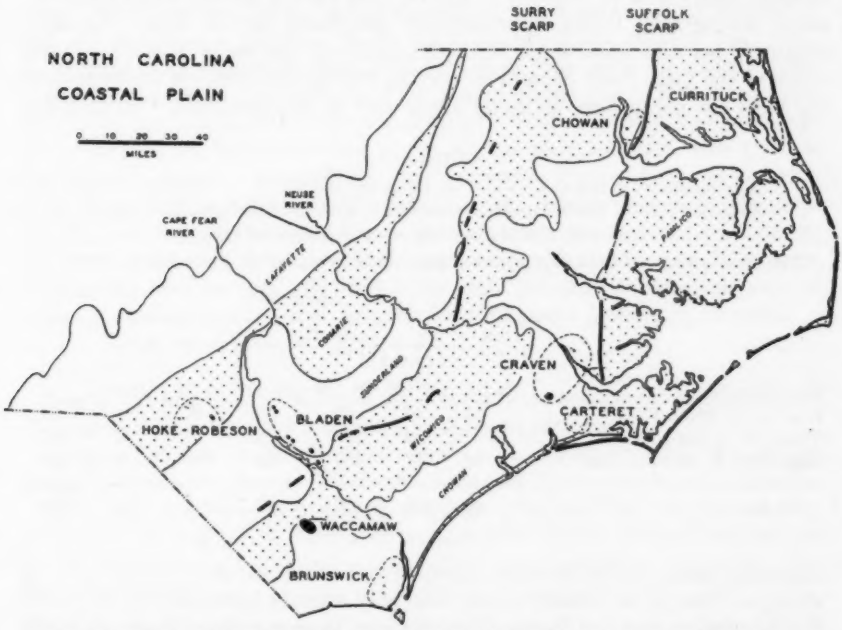

FIG. 1. Location of the Carolina bays sampled in 1949 in relation to Coastal Plain terraces and wave-cut scarps. The terraces are taken roughly from Clark *et al.* (1912) and the location of the scarps from Flint (1940).

of equilibrium phenomena resulting from various sea levels should probably be discarded, but in the present paper the original designations will be retained primarily as a convenience for designating general zones of elevation. The two scarps are shown in Fig. 1.

DISTRIBUTION OF CAROLINA BAYS

A second disputed question, which is even less close to being settled than the first, concerns the origin and subsequent history of the very numerous elliptical basins—the Carolina bays—in the Coastal Plain. Theories concerning their origin are quite numerous and have been reviewed by Johnson (1942), but studies concerning the bays themselves have been altogether too few, so that at present

there is no conclusive evidence compelling the acceptance of one or another of these theories.

On the basis of a map showing the distribution of all bays larger than 800 ft. compiled from aerial photographs by the late W. F. Prouty and the terrace map by Clark *et al.*, a count was made for each of the terraces in each of three transverse portions of the Coastal Plain (Table 1).

The bays are not distributed uniformly over the state, as was obvious previously from Fig. 2 of Johnson (1942) or from even a cursory examination of aerial photographs. Of the approximately 2000 large bays in North Carolina, almost 90 per cent occur south of the Neuse River, and more than 50 per cent south of the Cape Fear. Moreover, even in the areas of greatest concentration the bays are not present in equal abundance at all elevations. Omitting the

TABLE 1

Approximate number of Carolina bays greater than 800 ft. in diameter in the Coastal Plain of North Carolina, arranged according to the terraces of Clark et al. (1912) and according to three transverse belts

The counts were made in part from an unpublished map by W. F. Prouty.

REGION	TERRACE						TOTALS
	Lafayette	Coharie	Sunderland	Wicomico	Chowan	Pamlico	
Elevation, ft.	>235	235–160	150–110	100–50	50–25	25–0	
Va. line to Neuse River	13	40	45	33	43	30	204
Neuse R. to Cape Fear R.	2	188	185	178	74	4	631
Cape Fear R. to S. C. line	10	577	380	22	176	0	1165
Totals	25	805	610	233	293	34	2000

Lafayette terrace which has only a few, some of which are doubtful and most of which are close to the Coharie terrace and may actually be situated on the latter through error in locating the boundary between these two zones, there is roughly a decrease in number of bays with decreasing elevation. Thus the highest terrace, the Coharie, has the greatest number of bays and the Pamlico the least. Assuming that there is a concentric chronological arrangement of surficial Coastal Plain sediments corresponding to the terraces, one is tempted to speculate that the older a formation is the more bays it has. A corollary of this is that the bays are not necessarily all the same age.

PRESENT STUDY: METHODS

In an attempt to gather data concerning the comparative age and ontogeny of basins located at various elevations, a study of the sediments of the Carolina bays which was started on Singletary Lake in 1948 was extended to a number of diverse bays in 1949. Fig. 1 shows the bays and areas sampled in relation to the Coastal Plain terraces and the Surry and Suffolk scarps.

In each area studied several bays were sampled to provide a more nearly repre-

sentative picture than could be obtained from just one bay and to give some idea of possible variation with respect to size. The bays were selected for their regularity of outline so that there would be no question as to their being bays, for the presence of standing water or at least saturated conditions to provide continuous deposition and some protection from burning, for their general accessibility, and for a range of size. With the aid of aerial photographs and a sighting compass (and a machete!) the bay was penetrated to its approximate center. Along the way "soundings" were taken at regular intervals by means of the $\frac{3}{8}$-inch steel rods of the Davis peat borer. The total depth to which the rods could be pushed and any pronounced variation in the configuration of the bottom was noted. If the density of vegetation permitted, soundings were taken in various directions from the center to determine if the place selected was in fact the deepest part of the bay that could be conveniently found. When the sampling station was finally decided on, samples of the bay sediments were collected with a Davis peat sampler at 6-inch intervals (or sometimes more frequently as the occasion demanded) from the surface of the bay down as far as the sampler could be pushed and pounded without damaging it unduly. No attempt was made by other means to penetrate the sand or clayey-sand below this level. Sometimes a Hiller-type sampler was used for very soft surface deposits. The samples were preserved in properly labelled plastic screw cap vials for subsequent laboratory analysis.

RESULTS

This report presents just a few of the more general results and a suggestion of what may be expected from a complete analysis of the cores.

Bays are much more uniform when seen from the air than when seen from the ground. From the air they are all ellipses of varying size, oriented roughly parallel to one another in a general northwest-southeast direction. From the ground they are seen to have differing degrees of wetness and different plant communities. The latter are controlled by the prevailing moisture conditions, particularly the length of time each year that the soil is completely saturated with water, by the nature of the soil profile and by the frequency and severity of burning. Wells (1928) has designated the three main types of communities that occur in these wet depressions as swamp-forest, shrub-bog, and savanna-bog. Rockyhock Bay (Ch-3), a good example of a swamp-forest, consisted of a high canopy of large white cedars, sweet bays, and maples, with little shrub understory. More open swamp-forests, such as Cu-1, contained pocosin pines, black gum, maple, sweet gum, and holly, and in addition an understory of cane, ferns, small sweet bay, and abundant sphagnum. Still others had a rather thick shrub understory, with of course a more open canopy. The shrub-bog community, e.g. that of Cr-3 and Br-2, consists primarily of broadleaved evergreens, which in these instances were mainly waist-high to more than headhigh growths of gallberry (*Ilex*), pepperbush (*Clethra*), and titi (*Cyrilla*), frequently held together by bothersome cat briars (*Smilax laurifolia*), with many *Sphagnum*-filled pools below and scattered pocosin pines (*Pinus serotina*) projecting above this mass of vegetation. Other areas, such as extensive parts of Ca-1 and Ro-3, consisted primarily of grass and

TABLE 2

General geographic, morphometric, and edaphic characteristics of the Carolina bays of N. C. investigated in 1949

Lengths were measured without correction from USDA aerial photographs (scale approx. 1 in. = 1667 ft.), and altitudes were interpolated from U. S. Army Corps of Engineers maps, except for Waccamaw and Rennert which were obtained from N. C. Geol. and Econ. Surv. Bull. 27. Elevations of Singletary and Jones were reported previously by Frey (1949a). The elevation of RO-1 was interpolated from a nearby bench mark.

TERRACE	COUNTY	BAY	NAME	APPROX. ELEV.	LENGTH	DEPTH	SEQUENCE OF SEDIMENTS
				ft.	*yds.*	*ft.*	
Pamlico	Currituck	Cu-1		10	800	6.0	Thin organic, sandy clay, sand
		Cu-2		10	1060	6.0	Organic, clay, alternating sand and clay
		Cu-3		14	700	4.6	Organic, clay, alternating sand and clay
Chowan	Chowan	Ch-1		41	1030	5.0	Organic, brown sand
		Ch-3	Rockyhock Bay	18	1810	14.3	Organic, clay, organic, sand
	Craven	Cr-1			1150	10.0	Organic, clay, sand
		Cr-2			460	4.4	Organic, sand and clay, sand
		Cr-3			1040	5.8	Organic, sand
		Cr-4	Catfish Lake		3020	6.5	Organic, clay, sand
	Carteret	Ca-1			640	4.5	Organic, sand
		Ca-2			530	8.3	Organic with 2 sand lenses, clay, sand
		Ca-3			950	3.8+	Sandy organic
	Brunswick	Br-1		42	670	4.5	Organic, sand
		Br-2		48	630	4.2	Organic, sand
		Br-3		44	210	2.0	Sandy organic
		Br-4		52	480	4.3	Sandy organic
		Br-5	Funston Bay	45	1960	5.5+	Organic, clay, sand
		Br-6	Pumping Pond		440	16.0	Organic, sand
	Columbus	Wa-1	Lake Waccamaw	41	10820	14.7+	Organic, sandy clay, blue clay
Wicomico	Bladen	Si-25	Singletary Lake	63	3180	21.6	Organic, clay, organic, clay, organic, sand
Sunderland		J-1	Jones Lake	73	2590	17.7	Organic, clay
		Sa-1	Salters Lake		2660	18.4	Organic, clay, organic, sand
		LS-2	Little Singletary		3190	12.3	Organic, clay, organic, silt, sand
		Wh-1	White Lake		3740	12.4+	Organic, blue clay
		Bl-1			930	8.3	Organic, clay, sand
		Bl-2	Jerome Bay		2170	11.0	Organic, clay, sand
Coharie	Hoke	Ho-1			1040	3.5	Thin organic, clay, sandy clay
		Ho-2			740	3.0	Thin organic, clay, sandy clay
		Ho-3			940	5.4	Thin organic, clay
	Robeson	Ro-1		205	580	1.5+	Thin organic, sandy clay
		Ro-2			1020	2.5+	Thin organic, clay
		Ro-3	Rennert Bay	182	4580	7.6+	Organic, clay, sandy clay

ferns. Drier areas of some of the upland bays contained large holly and oaks. Evidence of fires was found in many of the bays.

The sediments occurring within the bays constitute the main material for further work (Table 2). All the bays had a layer of organic matter (ooze, peat, or organic soil) at the surface. This varied in thickness from a few inches in most of the bays examined in the Hoke-Robeson region to a number of feet in most of the other regions. Below this was an inorganic layer consisting of sand in the shallower bays and clay or silt in the deeper bays. The clay (silt) usually rested upon sand except in a number of the Bladen County lakes (Salters, Little Singletary, Singletary) and Rockyhock Bay (Ch-3) where a second organic layer was interposed. In core Si-25 of Singletary Lake two such organic layers were found, making a total of 3 including the now accumulating surface sediment. With the equipment at hand it was not possible to push down into the sand. This is unfortunate, since it has been found in the more extensive studies on Singletary Lake in the summer of 1948 when with the aid of a Wilson sampler samples were obtained well into the sand, that stratified pollen indicating basin deposition occurs in the sand as well.

With the understanding that the depths of sediments here reported constitute only those that could be sampled with the Davis or Hiller devices and do not necessarily represent the total thickness of sediments, it is interesting to examine the relationship between size of bay and depth of sediments. Fig. 2 shows that in general as the size of the bay increases, the depth of the sediments also increases. With the exception of Rockyhock Bay, the seven deepest bays are the ones still containing enough water to be called lakes. Rockyhock Bay is interesting in that it is the only filled-in bay sampled which shows any of the morphometric characteristics of the bay lakes (Frey, 1949a); at the southeast end is a shallow sand terrace extending out from the present shore of the bay for a considerable distance and then dropping off rapidly to the depth sampled. This greater depth of the lake-containing bays suggests that a greater original depth as well as their greater size may help account for open water persisting in these particular localities.

Pumping Pond in Brunswick County does not have the shape of a bay and is probably not a Carolina Bay. It is representative of the numerous small ponds found along much of the immediate coastal region of North Carolina, and is included for comparison with the bays. Likewise Catfish Lake is not a bay. The bottom of Catfish Lake was very irregular, with many "hillocks" of peat projecting above the clay surface of the bottom. In between was a soupy accumulation of flocculent organic matter, and in addition there were many logs on the bottom. There is a strong possibility that Catfish Lake has been reformed relatively recently by the erosion and removal of deposits which obliterated the previously existing water area perhaps during the climatic optimum. Jerome Bay (Buell, 1946) is the only other bay in North Carolina previously reported upon. It is likewise included for comparison.

Scattered references occur in the literature relative to the depth of bays and the nature of the contained sediments. Glenn (1895), to whom credit must be

given for first pointing out the bays and describing accurately some of their characteristics, noted that wells driven into bays showed a "dark fertile, compact clay, impervious to water" extending downward 15 to 25 ft. to the underlying water-containing sand. Johnson (1942) noted that Carolina bays may have a total depth up to 50 ft. below the general plain level. This, however, does not represent 50 ft. of sediments, since the floors of some of these crater-like depressions are themselves as much as 30 to 40 ft. below the surface of the surrounding plain. No such bays have been sampled by the author. On the basis of all existing information it is probably safe to conclude that the Carolina bays are

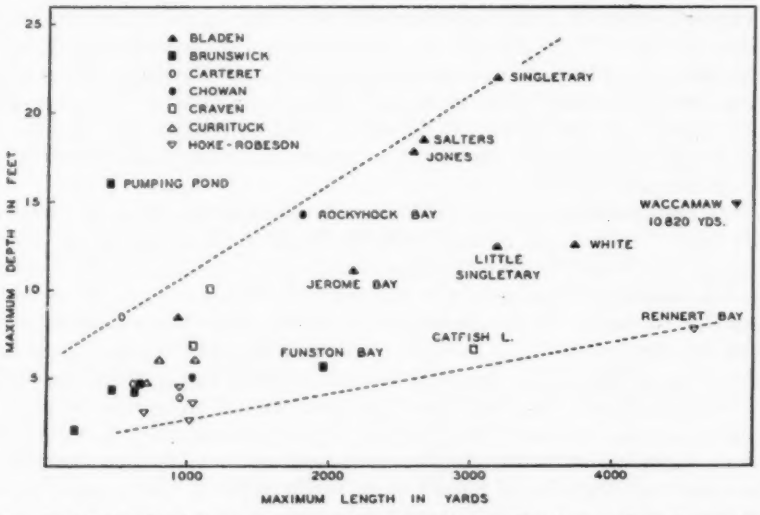

FIG. 2. Relationship between maximum length of the Carolina bays and maximum depth obtained with a ⅜-inch steel rod. Pumping Pond and Catfish Lake are not bays. The data for Jerome Bay are from Buell (1946).

quite shallow, much more so in general than the ice block basins or glacial erosion basins of the northern part of the continent.

ANALYSIS OF SEDIMENTS

The sediments of a marshy or water-filled basin, if not disturbed or severely desiccated and oxidized during their history, preserve a chronological record of plant and animal remains. Long term changes in climate are recorded by changes in the percentage composition of tree pollens in the sediments. Buell (1945) working on Jerome Bay in Bladen County was the first to observe and record spruce pollen from surface sediments of North Carolina. This type of tree, found only in the clay at the bottom of the core, along with a small pine grain identified as *P. banksiana* on the basis of size-frequency distribution, suggests these lowermost sediments were contemporaneous with late Wisconsin time. More recently

the author (Frey, 1949b) has analyzed core Si-25 containing the three organic layers. Although spruce is present in small percentages all the way to the bottom from where it first occurs in the upper part of the silt zone, there are definite ameliorations of climate associated with each of the two lower organic layers, as shown by increased percentages in oak, hickory, and even the occurrence of such pronounced mesophytic species as beech, chestnut, and hemlock. On the basis of these changes it was suggested that the sediments of Singletary Lake

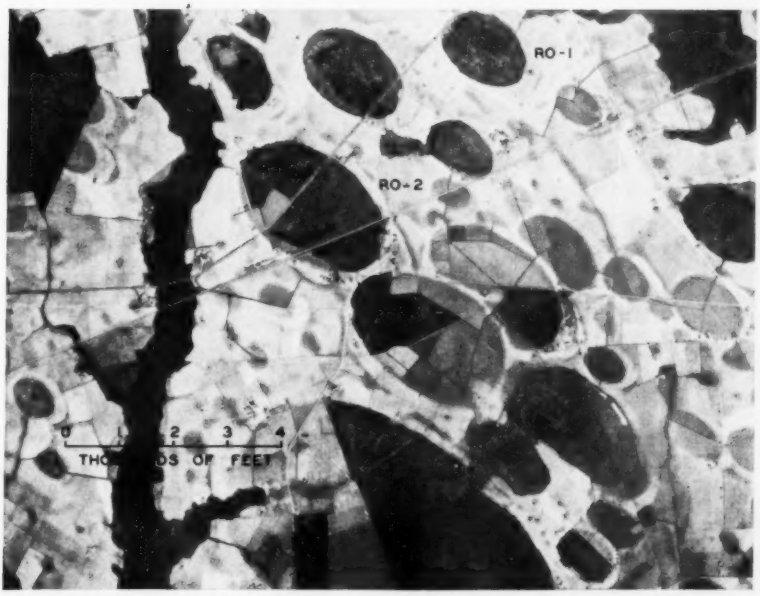

FIG. 3. Aerial photograph of a portion of Robeson County near Red Springs, showing the location of Ro-1 and Ro-2 and their relation to the surrounding surface features. The darker lines are drainage ditches. (USDA, Production and Marketing Administration.)

extend back into the Tazewell subage of the Wisconsin glacial age, making this record the longest continuous pollen record known from North America.

Certain regional differences are apparent in the bays which may be a reflection of differences in age. One of the most marked of these is that the bays on the Coharie terrace in particular are filled mainly with dark mineral soils having a relatively thin layer of organic matter on top. When these bays are cleared and drained they can be used quite successfully for agriculture. Fig. 3 is an aerial photograph of a portion of Robeson County near Red Springs taken on Feb. 20, 1938, showing the location of Ro-1 and Ro-2. The bays under cultivation can be readily distinguished by their darker color from the surrounding more sandy country.

Analysis of the cores collected from these and the other bays will enable the

aging of their sediments and the working out of their histories. When means is obtained of sampling the sand deposits—at least the pollen bearing portions—it should then be possible to determine quite readily whether or not all the bays are the same age. In the meantime the more superficial sediments on hand will be valuable in helping determine the influence of glaciation on the southern United States and possibly of following the retreat and advance of vegetations with changing climatic conditions.

SUMMARY

Although Pleistocene marine influence has not been demonstrated in the Carolinas at elevations greater than 100 ft., the peat-filled Carolina bays are more numerous above this level than below. There is a general decrease in number of Carolina bays with decreasing elevation on the several Coastal Plain terraces. The depth of sediments down to impenetrable sand or sandy clay roughly increases with increasing size of the bay. Those bays still containing lakes are in general the deepest, suggesting that an original greater depth of the basins may have helped in the preservation of the water areas. In the two studies thus far on the pollen of bay sediments, spruce has been found in the inorganic layers below the superficial organic layer. In Singletary Lake two lower organic layers were associated with ameliorations in climate, although these were not intense enough nor of long enough duration to cause a disappearance of the spruce. The usefulness of pollen analysis in working out the age and ontogeny of these basins and of studying changes in vegetation during Wisconsin time is indicated.

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THE HISTORY OF THE DISCOVERY (1600–1680) OF THE SPIRAL VALVE IN THE LARGE INTESTINE OF ELASMOBRANCHS AND A GANOID

By E. W. Gudger

American Museum of Natural History
New York City

INTRODUCTION

In the lower vertebrates, but especially in the Elasmobranchs, Ganoids, and Dipnoans, there is found that curious twisted outgrowth beginning anteriorly on the interior dorsal wall of the intestine and extending to the caecum, the spiral valve, similar perhaps to the typhlosole of the invertebrates (*e.g.*—the earthworm). In the fishes under study, it is an organ of many varying forms, but always fundamentally a spiral. Its function is to slow up the passage of food materials through the short intestine and by its greatly increased and highly vascularized surface, to promote absorption of the digested food. This is necessary because these fishes lack the long convoluted intestine of the higher animals in which absorption is facilitated.

I have long had it in mind to trace the history of the discovery of this very interesting structure. Knowledge of the relatively early investigation of the spiral valve in Elasmobranchs first came to me while serving as editor of Vol. III of the Dean Bibliography of Fishes. In 1921–22, while working on the Pre-Linnaean Section therein, references to Steno and Perrault were found and listed. Later the Museum Library procured copies of Adelmann's Fabricius and of Maar's Steno. Then I found Severinus in the Surgeon General's Library in Washington, D. C., and had a photostat copy made for our Library. But study of all these led to the belief that there must be other old anatomical works having descriptions of spiral valves embedded therein. Thus matters stood until knowledge of the subject was much advanced by the publication in 1944 of Dr. F. J. Cole's outstanding "History of Comparative Anatomy." In this he reproduces a number of the old figures of the spiral valve and gives brief citations in his text, but, in his invaluable bibliography, he cites authors, titles, years, and editions. In addition to the data in his book, Dr. Cole has by letters and photographs of texts and figures greatly helped my work along.

But for this article I must have full citations and not all the works needed are available in our library. Furthermore, these descriptions, in these old books in which they are found, are "embalmed in that most excellent preservative—the Latin language." But alas my Latin is far in the past. However, help in this matter has come from Prof. Henry T. Rowell, head of the department of Latin in the Johns Hopkins University (my alma mater), who, with equal accuracy and kindness, has translated these excerpts for me. The help of both Prof. Rowell and of Dr. Cole is gratefully acknowledged.

With the kind aid thus given, I have endeavored to work the data on the history of the discovery of the spiral valve into a chronological narrative. But even with all this assistance the task has not been an easy one.

THE DISCOVERY OF THE SPIRAL VALVE IN ELASMOBRANCHS

As will be seen herein, the narrative of the discovery of this curious structure in various Elasmobranchs is a tortuous one. Great difficulty has been had in tracing the steps whereby each discovery was made and made known. These early anatomists did not always make full dissections of their sharks and rays and write clear descriptions of what they found, publication was difficult, and they did not always illustrate their books by drawings. Thus at least one early writer barely missed the discovery of the winding valve.

A "Near Miss", 1600

0.—Hieronymus Fabricius ab Aquapendente (1537–1619), an Italian who lived at a waterfall (as his cognomen indicates), published at Venice in 1600 a general study entitled "De Formato Foetu." Prof. H. B. Adelmann of Cornell University has published a facsimile edition (1942) of this work with a translation and with photographic copies of the original figures and copious critical notes. The "De Formato Foetu" has seemingly been published under various dates at various places. Adelmann used the fine copy in the College of Physicians of Philadelphia in which "the date, 1600, on the engraved title page has been altered by hand to 1606" (Adelmann, pp. 129–130). This copy was accompanied by thirty colored plates and ten leaves of printed explanatory matter. A short section of this book is "A Dissection or Description of a Pregnant Shark" (*Piscis galeus—Mustelus vulgaris* of the present day). This is found on pp. 373–375 of Adelmann.

The figure of the shark with the abdominal wall split and spread apart to show the viscera, is portrayed in the author's Tab. XXXI, Fig. LXIX (Adelmann, p. 571). It is reproduced herein as Fig. 1. Here we see, in the lower right side of the abdomen, the hinder intestine (his "F. intestinum colon"). On the exterior are plainly shown in the intestinal wall the bases of the turns of the spiral valve. Had Fabricius split open the intestine, he would have seen and surely have figured the winding valve. This failure is explicable only by reason of the fact that he was preoccupied with the matter of viviparity in his shark. His next figure (Tab. XXXII, Fig. LXXI) portrays the opened abdomen of the shark with the split-open uteri in situ showing the foetuses within. He also figures an excised and opened uterus with the young.

So far as this search reveals, Fabricius is the first to attest by drawings (non-placental) viviparity in a shark. But he never knew how narrowly, by failure to dissect the intestine of his shark, he missed making the initial discovery of the spiral valve. The best that can here be said for him is, in the phraseology of the airplane bombers of World War II, that he made a "near miss."

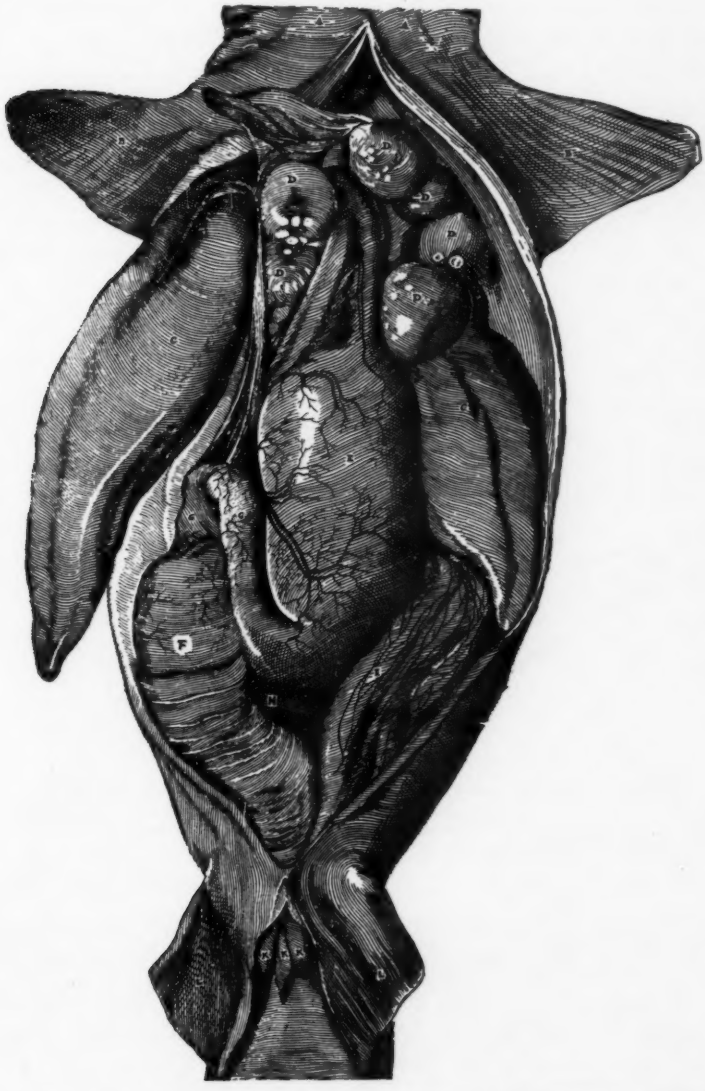

FIG. 1. An excellent drawing of the split-open abdomen of *Galeus laevis*, showing the viscera. Note on the lower right the undissected "intestinum colon" (F) showing the bases of attachment of about 12 turns of the spiral valve. The hinder turns are not clearly shown. After Fabricius, 1600.

The Discoverers, 1645–1680

The findings of these men will be presented in chronological order even though the author's name and second discovery must in some cases follow that of another anatomist.

I. (1, 2)—Marcus Aurelius Severinus[1] (1580–1656), also an Italian, published his "Zootomia Democritea," Noribergae (Nuremberg) in 1645. On p. 127, he presents a drawing (Fig. 2 herein) showing the excised hinder intestines of two Elasmobranchs. These are labelled "Canicula Aristotelis" and "Torpedinis."

FIG. 2. A diagrammatic drawing of the excised but undissected hinder intestines of Canicula Aristotelis and Torpedo showing the bases of the spiral valve in each.
After Severinus, 1645.

The spiral lines on the exterior of each intestine plainly show the basal outgrowths of the winding valves from the interior of the intestinal wall, but nowhere does Severinus refer to these. However, three lines above the figure, he says that—"In the intestine of both Raja & Galeus is found an admirable structure noticed by neither Rondelet nor Aquapendente." This presumably refers to the spiral valve, but Severinus neither figures nor describes at this point this "admirable structure."

On page 357, Severinus portrays in external aspect the entire digestive tract of

[1] The Roman numerals indicate epochs of discoveries of spiral valves and the men who made them. The Arabic numerals indicate the number of discoveries reported by the investigator at this point. They are cumulative—thus Steno II (3, 4). Swammerdam's ganoid discovery is set apart from his and other elasmobranch discoveries.

a Galeus excised and drawn in its entirety. This is reproduced as Fig. 3 herein. On p. 358 he says (translation by Prof. Rowell)—"The intestine of a Galeus of the first species is short and large. Its exterior is the color of blood. A double vein enters it from above, and above and below are three oblique twigs or sprouts [surculis] corresponding to each other, under which in the interior there are rather wide little membranes twisted into spirals to delay the feces." Now if for "obliquis surculis" we could read obliquis circulis—"oblique circles . . . under which in the interior there are rather wide little membranes twisted into spirals," the description would exactly fit the drawing (Fig. 3). It seems that here there is an undoubted typographical error.

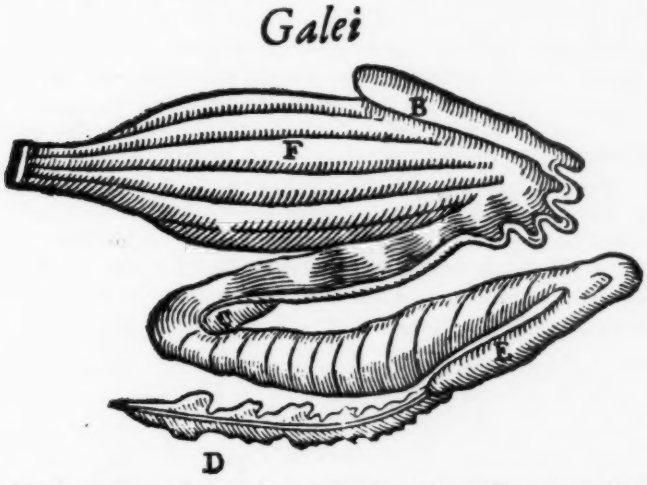

FIG. 3. The digestive tract of a Galeus removed and drawn. Note the 12 oblique turns of the bases of the undissected spiral valve in the hinder intestine.

After Severinus, 1645.

In Severinus' next paragraph, we read—"In the middle of the straight intestine a circle ["circulus"] of membrane is fixed, turning towards the rear. Its purpose, obviously, is to prevent [slow down] the escape of the juice [digested material]." It is significant that here circulus is used and not surculus. Surely surculus in the paragraph above is a typographical error for circulus.

On p. 371, Severinus begins a section headed "In Torpedine." At the end of this (p. 372) is found this statement—"The colon is large, but a little smaller than the ventriculus [little stomach]. The lower intestine is ingeniously equipped with coracoid [?] coils ['spiris coracoidis'] on its inner surface such as I have elsewhere mentioned for Raja, Caniculi [dogfishes], and Lupus [?]."

Even though Severinus did not figure a split-open elasmobranch intestine to show the winding valve, he portrays three intestines in external view showing the bases of the valves. These figures and his description of two valves (especially

when the presumed typographical error is corrected) do make it clear that Severinus saw this structure and recognized its function. Hence Severinus must be declared the Discoverer of the Spiral Valve in Elasmobranchs.—But, as will be seen, the next old anatomist to be quoted did a progressively better job.

Nicolaus Steno (1638–1686), or Niels Steensen in his native Danish, is the early anatomist whose successive descriptions and figures leave no possible doubt that he dissected, saw and understood the function of the winding valve in the intestine of several elasmobranchs.

Steno's collected works were published in two volumes (in one) at Copenhagen in 1910. His books and articles were edited with copious valuable notes by Vilhelm Maar. The printing is in good clear type and the figures are admirably reproduced. But unfortunately the editor did not put at the foot of the photographic reproduction of the original title page of each article the place and date of its original publication. These omissions surely impair the value of this fine piece of work. But I have, so far as possible, supplied them with a pen in our copy. What follows is from Maar's edition.

II. (1)—Steno in 1664 finished his "De Anatome Rajae Epistola" and dedicated it to Willem Piso (of Brasilian natural history fame). It seems to have been first published separately in 1664, and then republished in his "De Musculis & Glandulis Observationum Specimen" at Hafnia (Copenhagen) in 1664. Our interest is in the intestine and its valve. Here follows Prof. Rowell's translation.

Steno says [Maar, I, p. 199] that:

> The inner surface of the intestine of the ray is covered by its own [mucous] coat, which, however, was not as thick as that of the small stomach (ventriculus). If you turn it over undissected, you will see standing out from the coat directly behind the pylorus a sort of fibrous material which has the exact form of a spiral and descends to the region of the caecum. . . . There are three series of fibers in this snail [valve]. Two on the outside ascend at a slant from the covering of the intestine to the edge [of the valve?]; the one in the middle runs lengthwise but not in a straight line. Not far from the end of this snail-like layer is seen the exterior of a sort of sinewy base. It is white and ends in threads. I think that these threads are nothing but its fibers gathered together there and twisted toward what is below. This body or region [the space between the spiral markings?] is not small. It is two or more fingers wide and wraps itself eight times around the intestine. . . . Thus in a very short intestine, the coils have the appearance of a snail's [shell] coils, which [coils] very long intestines make in other animals.

Here then is the evidence that Steno dissected the intestine of a ray and saw and described the spiral valve therein and understood its workings (ante 1664 —the date of publication). It is very unfortunate that he did not have a drawing made.

II. (2)—Steno in 1667 published in Florence his "Elementorum Myologiae Specimen," etc. One of the component parts of this was the "Historia Dissecti Piscis ex Canum Genere "(*Scymnus lichia*). Of interest to us just here is what he

writes about the intestine (Maar, II, p. 152). "There were two notable things about this intestine. 1.—A muscular coat as thick [wide?] as a middle finger was composed of fleshy spiral fibers. 2.—It has a membranous snail [valve] which fills the entire cavity. The intestine itself was a span in length. The edge of the snail [valve] towards its axis was five spans long. The excrement in this snail [valve] had taken on a yellow color." Here again Steno unfortunately has no figure.

Later Steno dissected the embryos of two other sharks and figured their spiral valves. But chronologically he must be set aside for a few years to bring in another man who also in 1667 discovered a spiral valve in another shark and gave the best figure (1671) of any of the early anatomists.

In 1666 the Academie Royale des Sciences was constituted in Paris. It was shortly divided into sections according to the interests of its members. These met not for the reading of papers like our present day scientific societies but they were working groups (like our laboratory students) for the examination and study of specimens. The group of physicists, as the biologists were then called, was particularly interested in animal life. These men (presently to become known as the "Parisians"[2]) met for the dissection mainly of foreign animals that had died of disease in the Royal Zoological Park.

There is every reason to believe that Claude Perrault was the leader of the anatomists. He was a distinguished architect and physician—a man skilled in the handling of fine tools and in a knowledge of anatomy, who could (and presumably did) take the lead in the dissections and in the descriptions of the organs found—these being matters particularly in his field. There is every reason to believe that Perrault's text was read to and discussed and possibly amended by the company, but we must believe that the final text was mainly his work.

III. (1)—Claude Perrault (1613–1688) is by all hands pronounced the dissector and writer of "Observations qui ont este faites sur un grand Poisson dissequé dans la Bibliotheque du Roy, le vingt-quatriéme Juin, 1667." Herein first appeared the description of the spiral valve of the "grand Poisson" (*Alopias vulpes*), a thresher shark or "marin renard," but without a figure. This article on Alopias and the description with figures of the dissection of a lion, were published in quarto in 1667 as a brochure of 27 pages without title page. But with

[2] The Parisians, like many other students, started with small beginnings. Their first brochure of two articles was published without title page and in quarto size at Paris in 1667. By 1669, five more dissected animals had been added to the list. And in 1671, a volume in folio appeared under the title "Mémoires pour Servir à l'Histoire Naturelle des Animaux, Dressés par M. Perrault, etc." These Memoirs were illustrated with copper plate engravings. As Cole's bibliography (1944, p. 501) shows, the memoirs appeared with additions in many editions over many years, and formed a sure foundation for the study of comparative anatomy.

For an extensive and illuminating discussion of the "Parisians" and of their work and publications—and especially of Perrault's outstanding part therein—see Cole (1944, pp. 393–442). Cole has gone into bibliographic refinements, and has made bibliographers and librarians his debtor (1944, p. 501).

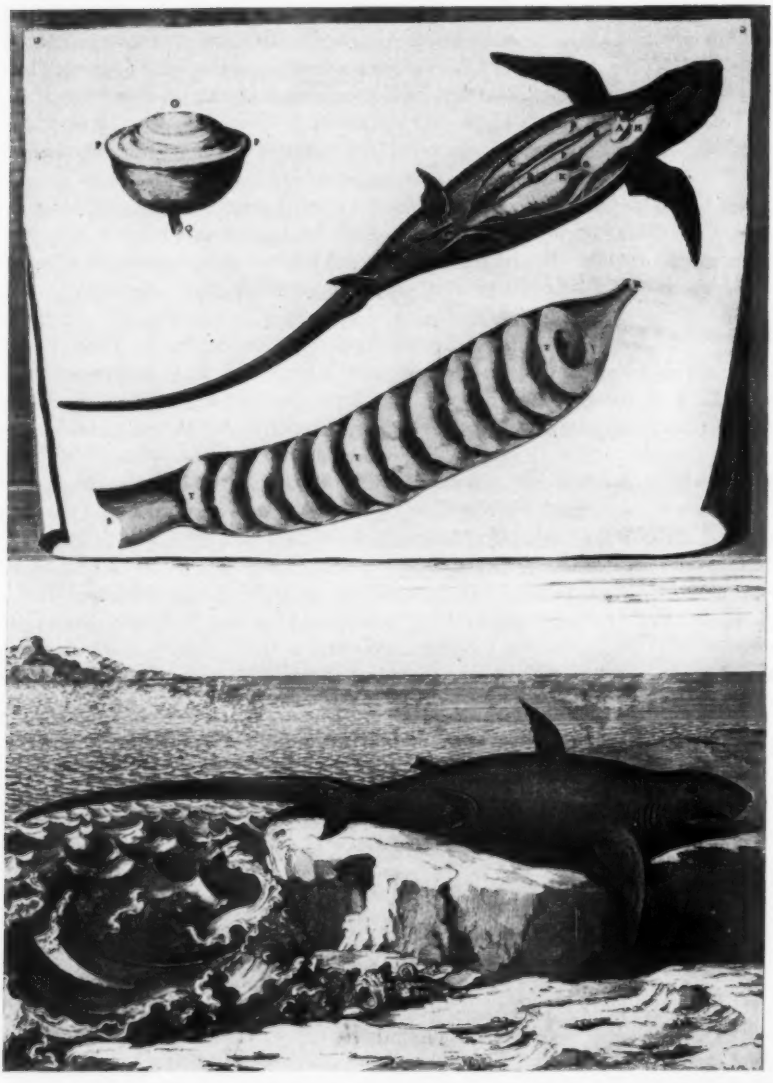

FIG. 4. Below is shown lying on a ledge of stone a "renard marin," a fox or thresher shark. At the top is seen the shark with abdomen slit open to show the viscera. Below it is the spiral valve of 12 coils with half the intestinal wall removed. Note the opening in the center of each coil. This is the first and best of the early published figures of the spiral valve of a shark.

After Claude Perrault, 1671.

the like work on other animals, the two articles above appeared in folio at Paris in 1671, bearing the title "Memoires pour servir à l'histoire naturelle des animaux." In this the figures for the "grand Poisson" were first published and 1671 is their date. Fig. 4 herein is copied from the 11 x 15½-inch original. It is the first figure of a spiral valve ever published. In later editions from 1676 on, other dissections and figures appeared in the growing "Memoires."

According to the description (1667), the "grand Poisson" was 8½ feet long. The large intestine was about 18 inches long and 3 broad. For 13 inches of its length (to the caecum), it had a peculiar structure. Instead of an intestine with the ordinary convolutions found in other animals, this was a straight tube whose cavity was interrupted by several separations composed of the lining membrane of the gut folded inward. These separations were about half an inch distant from each other and turned round like the shell of a snail or a staircase with an open center (one lacking a central supporting post). This valve, the description goes on to say, is the means by which food material is held back and is a great while in passing, although the way is very short.[3]

The drawing from the 1671 folio is reproduced herein as Fig. 4. Disregarding the eye in the upper left area, note that at the bottom the long-tailed shark is portrayed lying on a ledge of stone. Above is shown the shark with the abdomen slit open to show the viscera. Our attention, however, is centered on the figure of the large intestine which has been cut out and has had half of its intestinal wall removed to show the spiral valve in situ. Note the hollow center of the valve extending from anterior to posterior through the 12 coils of the valve. This finely portrayed valve is far beyond the crude figures of the other valves presently reproduced.

Now in turn we must leave Perrault behind (to be returned to in 1680) to bring forward two anatomists who published notes and figures of spiral valves in the early 1670's. The first of these is a newcomer, the Dutchman, Swammerdam, who produced a notable drawing with a very brief and inadequate description.

IV. (1)—Jan Swammerdam (1637–1680) in 1673 published in the fourth revised edition of Thomas Bartholin's "Anatome", issued at Lugduni Batavorum, two splendid figures of the spiral valve of a Raja (Fig. 5 herein). No. I in the figure is of the "Dissectum intestinum inversum" [turned inside out] and partially split. No. II is of the intestine with one-half of the wall removed to show the valve in situ. No. III is of the valve removed and drawn as a separate object. Note the slight perforation in the center of each of the turns.

[3] It should be said that no definite statement has been found in this study that Perrault dissected the shark and wrote the description of what was found. If all the editions and republications of this article (some 15 or 20 in number) could be examined, some light might be found to clear up the matter. But by common consent of bibliographers, librarians, and anatomists, the article is attributed to Perrault. At least one edition of the "Mémoires pour Servir" reads on the title page—"Dressez [arranged] par M. Perrault," and another "Redigez [edited] par M. Perrault."

In the first account of the spiral valve of a ray (1664), Steno gives a full description but no figure. In the ray under study, Swammerdam (1673) gives two good figures of the valve and almost no description. After noting that the intestine is turned inside out and partly dissected (I in the figure), he gives $2\frac{1}{2}$ lines to the valve as follows: "A valve like a snail [shell] in the intestine of the Raya is seen in figure II, as by one looking at it from the outside. It is seen in figure III with all its coverings (literally: tunics) removed."

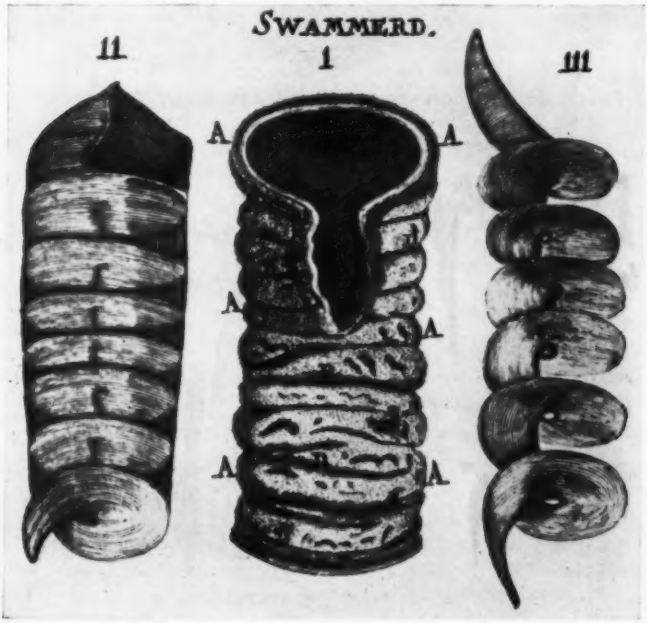

FIG. 5. Excellent figures of the spiral valve of a ray—the first ever published. I. shows a section of the everted and partly split intestine; II. is of the valve with half the intestine removed; III. portrays the valve removed from the gut.

After Swammerdam, 1673A.

Of the history of these drawings, it is simply noted [by Bartholin?] that Swammerdam had communicated them through "our common friend, G. Blasius." Bartholin's "Anatome" (1673) is a very rare book, and none of the early anatomists who worked on the spiral valve nor Dr. Cole at the time of the publication of his book (1944) knew of these figures by Swammerdam. Dr. Cole found them later, and to him I am indebted for photographs of the valve and of the text quoted herein.

Having considered Swammerdam's unique figure, we now return to our grand old Dane, Nicolaus Steno, who, in addition to his previous discoveries of the

spiral valve in 1664 and 1667, now adds two more Elasmobranchs to his list with figures of their valves.

II. (3, 4)—Steno in 1675 published in Copenhagen in Acta Medica & Philosophica Hafniensia his "Ova viviparorum spectantes observationes," in which he figured but did not describe the spiral valve in two shark embryos obtained by dissecting the oviducts of two pregnant viviparous mothers.

In *Galeus laevis* (the "hound"), he found three foetuses in each oviduct. One of these he dissected and on his p. 220 (Maar, II, p. 170), he portrayed the alimentary tract as removed from the body of the foetus. This is shown herein as Fig. 6. Steno speaks of the "cochleatum intestinum" but does not describe it. *Galeus laevis* is a placental viviparous shark (as Fig. 6 shows) and Steno was particularly interested in the manner of nourishing the embryo by this placental apparatus.

Steno was the first to figure in a shark the yolk-sac placenta and its yolk-stalk connection with the intestine of the foetus, but he was not the first to see it. Aristotle (384–322 B.C.), the great Father of Natural History, wrote of this apparatus as follows: "The young in the womb develop with the navel-string attached by a sucker to the womb, so that as the egg-substance gets used up, the embryo is sustained to all appearance just as in the case of quadrupeds." (Historia Animalium, VI, 10, D'Arcy W. Thompson trans., Oxford, 1910).

In Steno's day there was, of course, no knowledge of the osmosis of digested food materials from the blood-vessels of the uterine wall of the mother into the like vessels of the placenta of the foetus. However, he was the first to figure the placental yolk-stalk connecting the wall of the maternal uterus with the beginning of the spiral intestine in the foetus of a shark (Fig. 6 herein).

In the same article, Steno (1675) describes the dissection of another pregnant viviparous shark—his "Spina piscis" (*Acanthias vulgaris*) with young free in the oviducts (uteri). On one side he found one foetus, and on the other "gemini" (twins). He opened the abdomen of one of the "pisciculorum" and figured the digestive tract on his p. 203 (also in Maar, II, p. 172) (Fig. 7 herein), for comparison with that of his young *Galeus laevis*. The little dogfish drew its sustenance only from the yolk-sac (D). In some non-placental Elasmobranchs, after the yolk-sac is exhausted, the young are fed on a milk-like fluid secreted by the villous outgrowths from the uterine mucosa. Steno several times refers to the "cochleatum intestinum" of the two foetuses but nowhere describes it as shown in Figs. 6 and 7. Evidently the presence and function of the spiral intestine had been so established by him in a ray and in another shark that he concluded that it needed no further discussion here.

From Steno, with his high record of two descriptions without figures and two figures without descriptions, we now return to Perrault, who in 1680 figured and described an entirely new type of elasmobranch spiral valve—the "scroll" or cornucopia valve.

III. (2)—Perrault in 1680–88 published his "Essais de Physique, ou Recueil de plusiers traités touchant les choses naturelles" in 4 vols., 12 mo. Paris. This

work is so rare that the only copy in England is in the library of Trinity College, Cambridge, but Dr. Cole has an edition published in 1721. From vol. III of this, which is identical with the original edition, except in size, he has sent me a photograph of Perrault's figure XV (1680). This is reproduced as Fig. 8 herein.

In this figure, Perrault's No. I does not concern us. No. II is the spiral valve in the dissected intestine of the thresher shark, *Alopias vulpes*, which has been already reproduced herein as Fig. 4 (1671). For his No. III the description reads as follows: "Fig. III represents the intestine of another fish called Morgast,

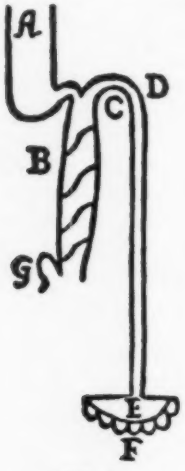

FIG. 6.

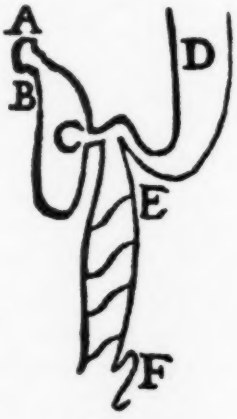

FIG. 7.

FIG. 6. A diagrammatic figure showing the ventriculus (A), the placenta (E & F), the yolk-stalk canal (D) emptying through the insertion (C) into the spiral intestine (B). G is the caecum. This is the earliest known figure of this placental apparatus in a shark.

After Steno, 1675.

FIG. 7. A diagrammatic drawing of the nutritive apparatus of a viviparous non-placental or yolk-sac-nourished shark embryo—*Acanthias vulgaris*. D is the yolk-sac and its tube—this opening into the spiral intestine (E). F is the caecum.

After Steno, 1675.

which is the *Galeus glaucus* of the ancients [the *Carcharias glaucus* of today]. It [Galeus] has also a single sheet of tissue very large and rolled as a horn [cornucopia] of small size. It is represented as being cut open obliquely to enable one better to understand its structure." This is the first published figure, so far as this search reveals, of a spiral valve in an elasmobranch other than a winding or screw valve without a central rod of support. And with this discovery of the scroll valve and the publication of its figure, the history of the discovery of the winding valve in sharks and rays may be considered ended.[4]

[4] The reader seeking to learn about the structure and development of the spiral valve in Selachians should consult R. Kantorowicz, Zeitschrift Naturwissenschaften, 1897, vol.

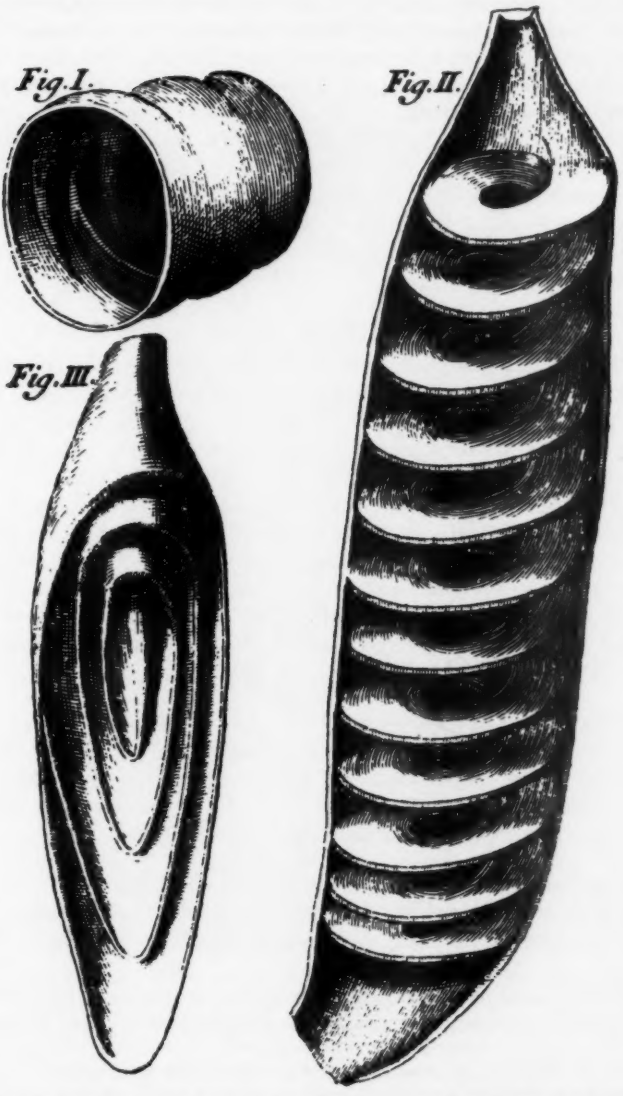

FIG. 8. Excellent drawings of the spiral valves of two sharks. Fig. I is of no concern to us. II is the spiral valve of the "marin renard" (*Alopias vulpes*). III is the scroll valve of the Morgast or *Galeus glaucus* (the *Carcharias glaucus* of the present day). This has been cut obliquely to show the winding structure.

After Claude Perrault, 1680.

About 1664, there was established in Amsterdam a fore-runner of the Paris anatomists and their "Academie" (1666). This was the "Collegium Privatum Amstelodamensis", which had a name but not a home. In 1667, the College published a little brochure, about $3\frac{3}{4}$ x $6\frac{1}{2}$ inches in size and with a total of 45 pages and 2 plates, under the title "Observationes Anatomicae Selectiores Collegii privati Amstelodamensis." In 1673, it completed the volume with 53 pages of text and 10 plates with the title "Observationum Anatomicarum Collegii privati Amstelodamensis, Pars Altera." This little book is so exceedingly rare that Reading University has reprinted a facsimile edition under the editorship of Dr. Cole. He has presented a copy of this to the Library of the American Museum of Natural History.

THE SPIRAL VALVE IN THE INTESTINE OF A GANOID

In 1673 there was published, in Pars Altera of the little book above noted, an unsigned article simply entitled "Acipenser." It and its two plates covered 5 pages of this diminutive booklet. Cole, who has gone into the matter more deeply than anyone else, attributes it to Swammerdam—who was the leader of the "Amsterdammers."[5]

IV. (2)—Swammerdam (1637–1680) gives a drawing (Fig. 9 herein) which is of particular interest because it shows the intestinal tract of the sturgeon with one wall removed (1673B). Here we see the "elegans Cochlea (H) in intestino tenui," or rather the remnants of the spiral valve left after dissection of the ventral surface of its coils. Note the "Scopus cochleae" (K), or central post of the valve —the first and only such "newel" post in any winding valve studied herein. The text (Prof. Rowell's translation) says that Acipenser has—

a rather thick intestine which is smooth on the outer surface. On the inside it is rough and uneven and covered with a granular layer (or crust). As the intestine comes to its hinder part, it has small valves or certain sinewy bodies at a distance of three fingers from each other. These cut across the intestine obliquely and thus give the appearance of some sort of a snail [shell].

So far as this research has found, this is the earliest figure (1673) of the spiral valve of a ganoid. The publication of it and its brief description establish the fact that this Ganoid—the only one found in Europe—has a spiral valve.

With the publication in 1673 of Swammerdam's figure and description of the spiral valve in a sturgeon, its presence in all ganoids is a strong presumption.

70, 337–364, pl. & 4 text-figs. There is no general work known to the present writer in which the various kinds of spiral valves in elasmobranchs are brought together and studied. The paper most nearly approaching such treatment is by T. J. Parker in Transactions Zoological Society of London, 1880, vol. 11, 49–61, 2 pls. (23 figs.).

[5] For the men who made these "Observationes," and published this little book, Cole suggests this group name—correlative with that of the "Parisians."

Furthermore, the presence of the valve found in various sharks and rays from 1600 (and particularly in 1645) to 1680, definitely established its presence in all

FIG. 9. The first published figure of the spiral valve of a ganoid—the dissected intestinal tract of a Sturgeon. Note particularly in the upper right the "elegans cochlea in intestino tenui" (H). The valve is largely dissected out but is represented diagrammatically by a winding dotted line. Also note the "Scopus cochlea" (K) or central post around which the valve winds. This is the first-known figure of a "newel post" spiral valve.

After Swammerdam, 1673B.

Elasmobranchs. The discoveries are put succinctly in the following Recapitulation, in which it will be seen that Severinus is the discoverer and that Steno and Perrault have done most to establish the presence in sharks and rays.

RECAPITULATION

There are recorded herein the discoveries of

NINE SPIRAL VALVES IN ELASMOBRANCHS

A "Near Miss"

0.—Fabricius (1600). Fig. 1.—Abdomen of *Galeus laevis* slit open to show the viscera. Intestine unopened but showing on the exterior the internal bases of the spiral valve. No dissection of the gut—a "near miss."

The Discoverers

I. (1, 2)—Severinus (1645). Fig. 2.—External view of hinder intestines of Canicula and Torpedo, each showing external markings of the internal valve. Fig. 3.—The whole intestinal tract of Galeus shown in ventral view. Markings on undissected hind gut are very clear. Valves not figured but described for Galeus and Torpedo (from other specimens?).

Severinus—the Discoverer.

II. (1).—Steno (1664). Spiral valve of Raja not figured but well described and action explained.

II. (2).—Steno (1667) did not figure but clearly described the spiral valve in a shark—"ex Canum genere" (*Scymnus lichia*).

III. (1).—Perrault (1667) described the winding valve in the hind intestine of a thresher shark (*Alopias vulpes*) and in 1671 illustrated his description by an admirable drawing (Fig. 4 herein). This is the first published drawing of a spiral valve of a shark.

IV. (1).—Swammerdam (1673A) published a plate of three figures (No. 5 herein) showing the intestine of a Raya: (I) turned inside out and partially split, (II) a spiral valve with half the intestine removed, and (III) the valve drawn separately. His description is very brief. These are the first published figures of the winding valve in a ray.

II. (3 and 4).—Steno (1675) portrays (the earliest figure) the placenta-yolk-stalk apparatus leading into the spiral valve in the gut of a foetal *Galeus laevis* (Fig. 6 herein). He also gives us another diagrammatic figure (No. 7 herein) of the winding valve in a foetus of a non-placental (viviparous) "Spina piscis" (*Acanthias vulgaris*). Neither valve is described.

III. (2).—Perrault (1680) figured and briefly described the "scroll" valve of a *Galeus glaucus* (*Carcharias glaucus*). This is the first dissection and portrayal (Fig. 8 herein) on record so far as has been found of a non-screw-shaped valve in a shark, and ends our series for sharks.

A SPIRAL VALVE IN A GANOID

IV. (2).—Swammerdam (1673B) portrayed and briefly described for the first time the winding valve in a Sturgeon (Ganoid). This (Fig. 9 herein) is the first valve to be found in this study to have a central pillar or support.

There are reproduced herein in external view or in dissection 10 figures of spiral valves—9 in Elasmobranchs and 1 in a Ganoid. In addition, reference is

made to descriptions (without figures) of two valves in a shark and in a ray, respectively, making a total of 12.

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STUDIES IN THE LEGUMINOSAE. V. CYTOLOGICAL OBSERVATIONS ON *CROTALARIA*

By Earlene Atchison

Department of Botany, University of North Carolina, Chapel Hill, North Carolina

Crotalaria L. consists of 250–400 species, depending on the authority, of annual and perennial herbs, subshrubs and shrubs, the definitive character being inflated pods. Various species are commonly known as "rattleboxes" in the southeastern states. The genus occurs throughout the tropical and warm temperate regions of both hemispheres; the greatest number and diversity of species seem to be concentrated in tropical Africa, with outlying centers of variation in Southeastern Asia and Central America. Various species are of some interest as forage and cover crops, alkaloid and fiber sources. Baker (1914) has reviewed the taxonomic history and monographed the African species; Senn (1939), the North and Central American species. There is no complete monographic study of the Asiatic group. A new, complete treatment of the entire genus would be difficult, but desirable.

The cytological literature on *Crotalaria* contains few detailed studies. Eichorn (1937) described the mitotic cycle in *C. fulva* and *C. spinosa*. Kawakami (1930), Hagerup (1932), Ramanujam, Parthasarthy and Ramiah (1933), Breslavetz, Medwedwa and Magitt (1934), and Senn (1938) have reported chromosome numbers for various species. Sundar Rao (1943) (taken from Darlington and Janaki-Ammal, 1945) reported $n = 8$ for 10 species. Raghaven and Venkatasubban (1943), in a study of the unrelated Zingerberaceae, reported unpublished work on 5 species of *Crotalaria* which had been observed to have identical number and morphology. These lists, although covering 19 widely distributed species, contain only one number other than $n = 8$, *C. incana* with $n = 7$.

Cytological and growth habit studies for 16 additional species are included in the present paper.

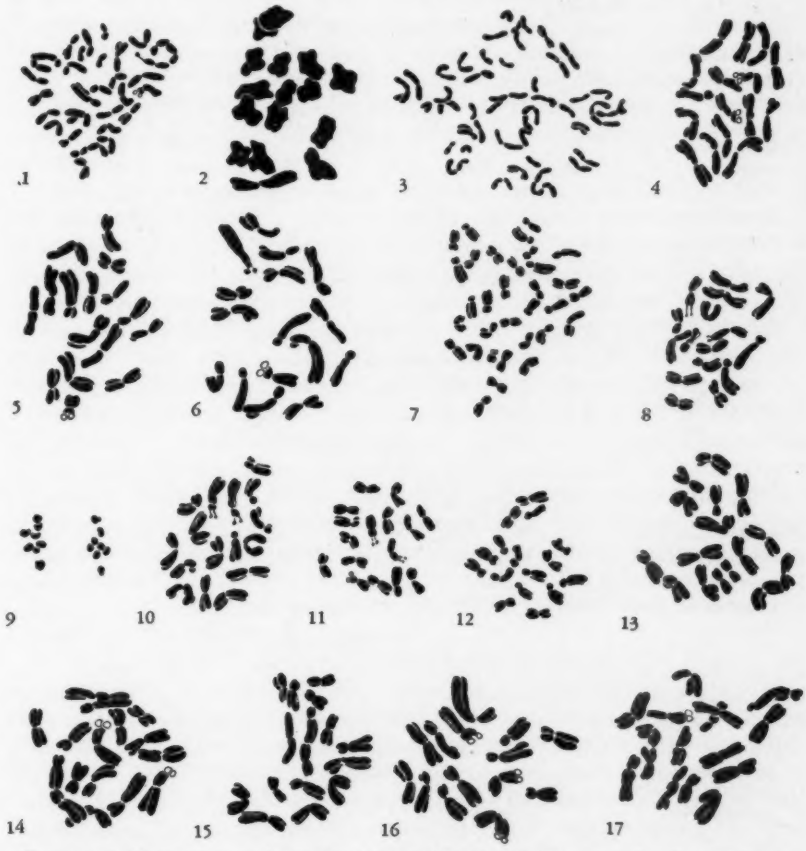
Chromosome number determinations (Figs. 1–17) have been made from acetic orcein leaf smears. Young leaves were prefixed for one hour in a saturated solution of paradichlorobenzene (Meyer, 1945). Meiosis was studied from direct acetic orcein smears of pollen mother cells.

Species were grown in experimental plots at The Blandy Experimental Farm, Boyce, Virginia, and at the Atkins Garden and Research Laboratory, Cienfuegos, Cuba.

General distribution, chromosome numbers, previous citations, and the present author's collection number are indicated in Table I. Where possible the species in Table I are grouped into Sections according to Baker (1914) and Senn (1939). Because of the general inaccessibility of cytological studies on *Crotalaria*, previous reports are summarized in the table.

The Asiatic, African, and South American species are principally diploids,

$2n = 16$, $n = 8$ chromosomes. Exceptions to this number are found in *C. incana*, $2n = 14$, from the world tropics, *C. pumila*, $2n = 32$, from South, Central and North America, and *C. pilosa* and its var. *Skutchii* from Central America. Other



FIGS. 1–17. Chromosomes of *Crotalaria*. Magnification, ×1500, except Fig. 9, ×600.

1. *C. pilosa* var. *Skutchii*, $2n = 32$. 2. MI, $n = 16$. 3. Trichome, $4n =$ ca. 64. 4. *C. spectabilis*, $2n = 16$. 5. *C. verrucosa*, $2n = 16$. 6. *C. Saltiana*, $2n = 16$. 7. *C. pumila*, $2n = 32$. 8. *C. maxillaris*, $2n = 16$. 9. AI, $n = 8$. 10. *C. intermedia*, $2n = 16$. 11. *C. ivantulensis*, $2n = 16$. 12. *C. Grantiana*, $2n = 16$. 13. *C. usaramoensis*, $2n = 16$. 14. *C. goreënsis*, $2n = 16$. 15. *C. lanceolata*, $2n = 16$. 16. *C. incana*, $2n = 14$. 17. *C. mucronata*, $2n = 16$.

$2n = 32$ species are found in the limited section of the genus which has extended into temperate North America; these will be discussed in a later paper.

C. incana apparently had an African center of origin; 52 of its congeners are listed from Angola alone. Senn (1938) suggests that $n = 7$ in this species arose

TABLE 1

Chromosome numbers in Crotalaria

SPECIES	DISTRIBUTION	n	2n	AUTHOR AND COLL. NO.
Sect. I. Simplicifoliae Benth.				
C. pilosa Mill.	W.I., C.A., S.A.		32	Atchison, 341
C. pilosa Mill. var. *Skutchii* Senn.	W.I., C.A., S.A.	16	32	Atchison, 340
C. Arenaria Benth.	E. Africa	8		Hagerup, 1932
C. spectabilis Roth.	Asia		16	Atchison, 327
C. juncea L.	Asia-Australia	8		Ramanujam *et al.*, 1933
			16	Breslavetz *et al.*, 1934
C. alata Ham.	Asia	8		Kawakami, 1930
C. retusa L.	cos. Trop.	8	16	Kawakami, 1930
		8		Ramanujam *et al.*, 1933
C. verrucosa L.		8		Ramanujam *et al.*, 1933
C. verrucosa L.			16	Atchison, 70
Sect. IV. Farctae Benth.				
C. Saltiana Andr.	E. Africa		16	Atchison, 313
Sect. V. Spinosa Bak.				
C. spinosa Hochst.	E. Africa		16	Eichorn, 1937
Sect. VI. Eucrotalaria				
C. pumila Ortega.	S.U.S., C.A., S.A., W.I.		32	Atchison, 339
C. anagyroides HBK.	W.I., Mex., S.A.	8		Kawakami, 1930
C. maxillaris Klotz.	E. Africa	8	16	Atchison, 303
C. intermedia Kotschy.	Africa		16	Atchison, 333
C. ivantulensis Welw.	Africa		16	Atchison
C. Grantiana Harms.	Africa		16	Atchison, 323
C. Dilloniana Baker.	Africa		16	Atchison
C. usaramoensis Bak. fil.	E. Africa	8		Kawakami, 1930
C. goreënsis, Guill. et Perr.	Africa		16	Atchison
C. lanceolata E. Mey.		8		Raghavan & Venkatasubban, 1943
			16	Atchison, 325
C. argyraea ? Welw.	Africa	8		Senn, 1938
C. obovata Don.	Africa	8		Hagerup, 1932
C. quinquefolia L.	Asia		16	Sundar Rao, 1943
C. laburnifolia L.	Ind., Phil.	8		Ramanujam *et al.*, 1933
C. incana L.	cos. Trop.	7		Senn, 1938
			14	Atchison, 337
C. mucronata Desv.	cos. Trop.	8		Senn, 1938
			16	Atchison, 328
Sect. ?				
C. Valetonii Backer.	E. Indies	8		Kawakami, 1930
C. fulva.	Asia		16	Eichorn, 1937
C. usorensis.		8		Raghavan & Venkatasubban, 1943
C. breviflora.		8		Raghavan & Venkatasubban, 1943

through aneuploid reduction from an $n = 8$ ancestral form. Comparison of the chromosome morphology of *C. incana* with that of *C. mucronata* (Figs. 16 and 17), a closely related species, suggests that this reduction in number may have involved the loss of one pair of short chromosomes with median centromeres. *C. incana* contains 3 pairs of long chromosomes while *C. mucronata* contains only 2; *C. incana* contains only one pair of short chromosomes while *C. mucronata* contains 2. Such a reduction could occur through reciprocal translocation involving the loss of a centromere without loss of essential genetic material. Such translocations have been established as evolutionary mechanisms by Tobgy (1943).

C. pumila shows a wider tolerance to environmental variations than most of the related species. It is a weedy colonizer from warm temperate Argentina through the American tropics into warm temperate United States. In spite of this wide north-south distribution, however, *C. pumila's* limitation to this hemisphere suggests its origin as recent in comparison with its tropical relatives. The nearest morphological relative among the author's collections is *C. maxillaris* from east Africa. Pod development was stimulated, but no fertile seed were matured by the cross *C. pumila* x *C. maxillaris*. There is little intraspecific variation in *C. pumila* as might be expected if the species were of hybrid origin. The only character with notable variation is pod number per raceme: Three seasons of experimental culture show that some collections constantly mature 3 pods on each raceme while others mature 4 to 8. It is possible that the positive qualities of a short season reproductive cycle, resistance to the *Crotalaria* moth, and prolific production of fruit are effects of gene duplication through increased chromosome number.

Neither *C. incana* nor *C. pumila* shows sufficient morphological divergence to be separated from Section Eucrotalaria.

Polysomic cells with ca. 64 and 90 chromosomes (Fig. 3) were found in the trichomes of leaves of 7 plants of *C. pilosa* var. *Skutchii*. Not only are mitoses usually absent from normal differentiated tissue, but this material had not been pretreated with either paradichlorobenzene or any other substance which might have had a c-mitotic effect. The author has no explanation of the stimulus for chromosome division in these cells, but suggests that the quantitative increase in chromatin, if not actual chromosome number, may be correlated with differentiation, as proposed by Huskins and Steinitz (1948). Castronova (1945) has encountered divisions with multiple chromosome numbers in seedlings of *Acacia*, also a legume, germinated under sterile conditions.

An inverse correlation between chromosome number and size is generally clear: Chromosomes of *C. incana*, $2n = 14$, are slightly larger than those of most of the $2n = 16$ species, while those of *C. pilosa* and *C. pumila*, $2n = 32$, are relatively smaller.

Cytological and morphological patterns among *Crotalaria* indicate that the genus is ancestrally tropical with a diploid number of $2n = 16$ chromosomes. Those species with variant chromosome numbers also show variation from the central group in either distribution or morphology.

Comparison of growth habits in tropical Cuba with those in the temperate United States discloses notable specific modification. *C. juncea*, *C. spectabilis*, *C. mucronata*, *C. incana*, and *C. usaramoensis* survive as herbaceous annuals in temperate regions, but become woody and shrubby if given the prolonged tropical growing season and higher minimum temperatures. *C. intermedia* is a perennial herb in either climatic region. *C. lanceolata*, *C. maxillaris*, *C. pumila* and *C. retusa* are annual herbs in either region. The capacity for formation of woody tissue seems to be characteristic of some species, with expression dependent on environment. In other species woodiness is apparently totally absent.

SUMMARY

Somatic chromosome studies are reported for 16 species of *Crotalaria* L. which are new to the literature. The most frequently observed number is $2n = 16$. This is accepted as the basic diploid complement, and it is suggested that morphological and distributional changes have accompanied aneuploidy in the case of *C. incana*, tetraploidy in the cases of *C. pumila* and *C. pilosa*.

Perennial and annual growth habit are apparently dual capacities, with expression dependent on environment, in several species; in others habit is strictly limited by heredity.

Polysomic cells were observed in the trichomes of *C. pilosa*.

The author is grateful to Dr. Orland E. White for suggestions concerning the manuscript, and to the many individuals who have provided seed for these and subsequent studies.

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THE EFFECT OF A VITAMIN A DEFICIENT DIET ON THE NATURAL AND ACQUIRED RESISTANCE OF MICE TO INFECTION WITH *TRICHINELLA SPIRALIS*

By John E. Larsh, Jr. and Hazel B. Gilchrist

Department of Parasitology, School of Public Health,
University of North Carolina,
Chapel Hill, North Carolina

It has been demonstrated by certain workers (McCoy, 1934; Motomura and Umezu, 1936; Lawler, 1941) that animals placed on diets deficient in vitamin A show a lowered resistance to certain of the parasitic nematodes. McCoy was able to show the specific role of this vitamin in the development of resistance in rats to *Trichinella spiralis*. Young rats started on a vitamin A deficient diet when 25 to 27 days old showed a markedly lowered resistance to infection as early as 2 or 3 weeks. Those on the deficient diet also failed to develop any resistance to a second infection whereas the controls became immune.

The mouse has been shown to be a suitable host in experimental trichiniasis (Culbertson, 1942; Larsh and Hendricks, 1949; Larsh and Kent, 1949; Hendricks, in press; and others), but no reports were found dealing with the effect on their resistance of a vitamin A deficient diet. It was, therefore, the purpose of the present work with mice to determine the effect of such a diet on natural resistance and on resistance following repeated infections.

MATERIALS AND METHODS

The experimental methods for infecting animals and for recovering *T. spiralis* adults and larvae were the same as those reported in detail in an earlier paper (Larsh and Kent, 1949). The vitamin A deficient diet for the preliminary experiments was a rat diet used by Lawler (1941). This diet (Diet I) consisted of the following: casein (vitamin-test), 18.0 per cent; irradiated yeast, 5.0 per cent; corn starch, 67.4 per cent; cottonseed oil, 5.0 per cent; $CaCO_3$, 1.5 per cent; KCl, 1.0 per cent; NaCl, 0.5 per cent; $NaHCO_3$, 0.7 per cent; MgO, 0.2 per cent; and Fe citrate, 0.5 per cent. Fifteen drops of activated ergosterol (10,000 U.S.P. vitamin D units per gram) were added per kilo of mixture. The control diet was identical except for the addition of 1.0 per cent cod-liver oil. It was unsatisfactory to feed the diets in powdered form because of waste and contamination. To prevent this, plain bacto-agar (Difco) was melted and added to the powdered diets to make a mixture approaching the consistency of a solid stock diet. The details of this technique are as follows. All of the ingredients were carefully weighed and mixed thoroughly in the preparation of the powdered diets. The A deficient and the control diets were prepared separately, usually in 3 kilogram lots. For this amount, 60 grams of plain bacto-agar were dissolved in 2100 cc of distilled water in a large flask by autoclaving for 10 minutes. It was allowed to cool to a temperature of 56°C before being added to the dry ingredients. The

resulting mixture was kneaded thoroughly, then spread on a clean flat surface to a thickness of about one-half inch; and after standing for 20 to 30 minutes at room temperature, it was cut into squares and stored in a deep freezer. For ease of handling, the food was stored in paper bags containing 100 grams each.

The results obtained in the preliminary experiments following use of this rat diet were difficult to interpret. Therefore, it was thought advisable to repeat the work using a diet more suitable for mice. With helpful suggestions from Dr. H. P. Morris (personal communication) of the National Cancer Institute the following vitamin A deficient diet (Diet II) was formulated: casein (vitamin-test), 15.0 per cent; irradiated yeast, 5.0 per cent; cornstarch, 43.0 per cent; sucrose, 15.0 per cent; fat (Crisco), 18.0 per cent; and salt mixture (Jones and Foster, 1942), 4.0 per cent. The control diet was identical except that the amount of Crisco was lowered to 15.0 per cent and 3.0 per cent cod-liver oil was added. Because of a difference in consistency of Diets I and II, only 30 grams of bacto-agar dissolved in 1000 cc of water were needed for 3 kilograms of dry ingredients of Diet II. The preparation of this diet was the same as that described for Diet I except that it was necessary to refrigerate the diet for about one hour before it could be cut into squares.

The age of the mice at the beginning of the experiments varied from 3 to 5 weeks; the age for each experiment is given below. The mice were matched according to sex and approximate weight and divided into 2 groups, experimentals (on A deficient diet) and controls (on diet with cod-liver oil). In all cases the mice were allowed food and water *ad libitum*. The food was contained in wire feeding baskets suspended from the tops of the cages (Larsh, 1947), and sufficient amounts were added daily to prevent the accumulation of dried and hardened portions. A record was kept for each experiment of the weight change of each group of mice and of their daily food consumption from the beginning of the experiment until time of autopsy. Since these records showed no significant difference for the mice on the two diets, the data are omitted from the discussion of the experiments.

Student's "t" test was the instrument used to determine the statistical significance of the observed differences in numbers of parasites recovered from the experimental and control groups. A probability greater than 5 was not considered to be significant.

EXPERIMENTAL RESULTS AND DISCUSSION

I. Preliminary experiments to determine the effect of a vitamin A deficient diet (Diet I) on the natural and acquired resistance of mice to infection with T. spiralis

A. Natural resistance.—A series of 7 experiments was completed. All the groups of animals were 5 weeks old when put on the experimental and control diets, with the exception of those of experiment 7 in which the mice were 4 weeks of age. The animals were kept on the diets for varying lengths of time (from 6 weeks in experiments 1, 3, and 5 to 14 weeks in experiment 7) before receiving a single

infection. They were continued on the diets until the time of autopsy. The size of the infection varied from 300 larvae in experiments 1 and 2 to 900 larvae in experiments 5, 6, and 7. Seven days after infection the mice were killed and counts made of the adult *Trichinella* recovered from the small intestine. The results are shown in Table 1.

The difference in the number of worms recovered from the mice of the A deficient groups and control groups is not of statistical significance, except for experiments 1 and 5 (189.0 and 148.7, and 516.0 and 384.1, respectively). It is, therefore, difficult to interpret these findings. It is worth noting, however, that in experiments 2 and 6, similar to 1 and 5 respectively, there was no significant difference in the number of worms recovered, despite the fact that the mice were exposed to the deficient diet for 3 weeks longer than those of experiments 1 and 5.

B. Acquired resistance.—In this experiment, experimental and control mice were put on their respective diets when 4 weeks old and given an initial stimulat-

TABLE 1

Showing the numbers of adult T. spiralis *recovered from mice kept on an A deficient diet (Diet I) and from controls killed seven days after one test infection*

EXP. NO.	AGE WHEN PUT ON DIET	TOTAL TIME ON DIET	NO. LARVAE IN INFECTING DOSE	MICE ON A DEFICIENT DIET			MICE ON CONTROL DIET			PROBABILITY
				No. mice	Adults recovered		No. mice	Adults recovered		
					Ave. no.	Per cent		Ave. no.	Per cent	
	wks.	*wks.*								
1	5	6	300	6	189.0	63.0	6	148.7	49.6	$<.05$
2	5	9	300	7	191.4	63.8	6	145.7	48.6	$>.05$
3	5	6	600	6	305.7	50.9	6	280.7	46.8	$>.05$
4	5	9	600	6	318.3	53.1	6	308.8	51.5	$>.05$
5	5	6	900	7	516.0	57.3	7	384.1	42.7	$<.05$
6	5	9	900	7	434.3	48.3	6	379.8	42.2	$>.05$
7	4	14	900	11	321.0	35.7	9	341.3	37.9	$>.05$

ing infection 4 weeks later. Three additional stimulating infections of 300 larvae each were given at 2-week intervals. For each infection, 2 additional mice were given the infecting dose and killed 7 days later to check on the viability of the larvae used. In every case, the average recovery of adult worms from these viability controls was about that expected from the dose. The fourth stimulating infection was followed one week later with an infection of 900 larvae to test for the presence of resistance acquired as a result of the stimulating infections. This final infection is called the challenging infection. At the time of this infection, two groups of controls which were of the same age as the above mice and had been fed the A deficient and control diets but had not received the stimulating infections were also given the infection of 900 larvae. These mice were used to check on the effect of the diets on natural resistance, as in the previous experiments. One week after the challenging infection was administered, all of the animals were killed and counts were made of the adults recovered from the small intestines. The numbers of larvae from the muscles of the animals given the 4

stimulating infections were determined by dilution counts. The results of all of these counts are shown in Table 2.

TABLE 2

Showing the numbers of T. spiralis *adults and larvae recovered from mice killed 7 days after a challenging infection. The animals were kept on the diets from the age of 4 weeks until autopsy*

MICE ON A DEFICIENT DIET			MICE ON CONTROL DIET		
Mouse no.	No. adults in small intestine	No. larvae in muscles	Mouse no.	No. adults in small intestine	No. larvae in muscles
A. Mice given 4 infections of 300 larvae each at 2-week intervals followed one week later by a challenging infection of 900 larvae					
1	723	50,800	1	503	48,600
2	606	28,600	2	381	57,000
3	664	45,200	3	102	78,000
4	597	59,000	4	320	96,000
5	574	38,000	5	631	82,000
6	558	88,000	6	191	20,200
7	371	62,000	7	443	89,000
8	536	42,200	8	346	43,000
9	428	58,000	9	483	60,000
10	580	100,000	10	273	104,000
11	663	60,000	11	224	36,000
12	487	19,000	12	332	40,400
13	623	30,000	13	172	38,000
14	422	41,600	14	126	38,000
15	718	24,500			
16	508	26,000			
Ave. no.....	566.1	48,306		323.4	59,300
Percentage.....	62.9			35.9	
B. Mice given only the challenging infection of 900 larvae					
1	380		1	419	
2	308		2	337	
3	346		3	364	
4	375		4	336	
5	371		5	317	
6	275		6	335	
7	355		7	380	
8	176		8	264	
9	353		9	320	
10	305				
11	287				
Ave. no.....	321.0			341.3	
Percentage.....	35.7			37.9	

There is a significant difference in the numbers of adult worms recovered from the experimental and control mice which had been given the stimulating infec-

tions (A). Those on the A deficient diet averaged 566.1 as compared with 323.4 for the control mice. However, the difference in the number of adults from these controls (A, average 323.4) and from controls given only the challenging infection (B, average 341.3) is not significant. Therefore, no demonstrable resistance was developed as a result of the stimulating infections. This suggests that the significantly greater number of worms in mice of the A deficient group given these stimulating infections than in their controls was due to a breakdown in natural resistance. There is no explanation for the failure of the control mice to develop acquired resistance in this experiment, as previous work has demonstrated the acquisition of resistance in mice on a diet of Purina dog checkers (Larsh and Kent, 1949). This suggests that the rat diet used in the present work, although maintaining the mice in apparently good condition, may not have been satisfactory in supplying factors needed as a result of the stress of repeated infections. In this respect it is worth noting that McCoy (1934) was able to demonstrate in rats fed a similar diet that the controls became immune after two infections whereas those on the A deficient diet failed to develop resistance.

There is no significant difference in the number of adults recovered from the mice on the A deficient diet given only the challenging infection (B) and from their controls. Thus the natural resistance of the animals was not altered to a demonstrable degree by feeding the deficient diet for this period of time. This agrees with most of the experiments of the previous section (Table 1). On the basis of the present experiment, therefore, it would appear that the combination of the A deficient diet and repeated infections produced the breakdown in resistance reported above (A of Table 2).

The numbers of larvae recovered from the mice of the A deficient group (A, average 48,306) and their controls (average 59,300) do not differ significantly. Thus these results differ from those of adult counts in which the mice on the deficient diet harbored significantly greater numbers than controls. This could mean that the diet inhibited the production of larvae by the females in the intestine, or that resistance to the larvae during migration to, or life within, the muscles was not interfered with by this diet to the same degree as resistance against the adults. If the latter could be proved by further work, it would be of real interest in view of the fact that different antibodies have been demonstrated to operate against the adults in the intestine and the larvae in the musculature (Oliver-Gonzalez, 1941).

A final experiment was planned which was hoped would clarify certain of the inconsistencies of the above results. The most serious of these was the failure of control mice to develop acquired resistance (Table 2), a phenomenon demonstrated in previous work in mice on a different diet. The diet was changed from the above, used mostly for rats, to one more suitable for mice.

II. A final experiment to determine the effect of a vitamin A deficient diet (Diet II) on the natural and acquired resistance of mice to infection with T. spiralis

A. Natural resistance.—In this experiment, mice were weaned at 3 weeks of age, divided into two groups, and kept on their respective diets for 11 weeks

before being given a test infection of 200 larvae. One week later they were killed and counts made of the adult worms recovered from the small intestine (B of Table 3). There is no significant difference in the number of worms recovered from the experimental and control groups (average, 106.5 and 107.9 respectively).

TABLE 3

Showing the numbers of adult T. spiralis *recovered from mice killed 7 days after a challenging infection. The animals were kept on the diets from the age of 3 weeks until autopsy*

MICE ON A DEFICIENT DIET		MICE ON CONTROL DIET	
Mouse no.	No. adults in intestine	Mouse no.	No. adults in intestine
A. Mice given 2 infections of 200 larvae each at 4-week intervals followed 4 weeks later by a challenging infection of the same size			
1	77	1	79
2	65	2	52
3	60	3	68
4	55	4	54
5	50	5	48
6	88	6	54
7	80	7	76
8	71	8	67
		9	50
		10	73
Ave. no.....	68.2		62.1
Percentage.....	34.1		31.1
B. Mice given only the challenging infection of 200 larvae			
1	119	1	124
2	80	2	84
3	87	3	110
4	89	4	97
5	137	5	81
6	123	6	119
7	90	7	94
8	106	8	83
9	129	9	136
10	126	10	118
11	86	11	127
		12	122
Ave. no.....	106.5		107.9
Percentage.....	53.3		53.9

This is in agreement with the results of the majority of the preliminary experiments (Table 1). However, only 4 weeks on the same diet used in the above preliminary experiments were necessary to produce in young rats a lowered resistance to *Strongyloides* (Lawler, 1941). McCoy (1934) and Motomura and

Umezu (1936) also found that rats on a vitamin A deficient diet for a short time showed a lowered resistance to *T. spiralis*. This suggests a host difference in the effect of a vitamin A deficient diet on the natural resistance to *T. spiralis*.

B. Acquired resistance.—Three week old mice were weaned, divided into two groups, and fed their respective diets until the end of the experiment. When they were 5 weeks old, the mice were given an initial stimulating infection of 200 larvae, followed 4 weeks later by a second infection of the same size. In both cases, mice were included to check the viability of the larvae used. Four weeks after the second stimulating infection, a challenging infection of 200 larvae was administered. This was also given to two other groups of mice the same age on the same diets that had been treated as the above mice except that they did not receive the stimulating infections (B of Table 3). The mice were killed one week after the challenging infection and counts of adults made (A and B of Table 3).

The difference in the number of worms recovered from mice on the deficient diet (A, average 68.2) and from controls (A, average 62.1) is not of statistical significance. The mice on both diets had developed resistance to reinfection as brought out by comparing the numbers of worms recovered from them (A) with the much higher counts for the mice given only the challenging infection (B); the latter on the deficient and control diets, respectively, harbored an average of 106.5 and 107.9 worms. The probability of this difference occurring by chance is less than one in 100 similar experiments, hence highly significant. The development of acquired resistance here supports the above suggestion that failure to do so by controls in the previous experiment (Table 2) might have been due to inadequacy of the diet used. It is clear from the present results that the deficient diet as used did not interfere either with natural resistance or with the development of acquired resistance.

The findings of this final experiment, therefore, show that the need for vitamin A in the operation of resistance to *T. spiralis*, if such need exists, is certainly not of the same magnitude for mice as demonstrated earlier for rats.

SUMMARY

Preliminary experiments were performed in an attempt to determine the effect of a vitamin A deficient diet (Diet I), designed primarily for rats, on the natural and acquired resistance of mice to *Trichinella spiralis*. The results of most of the experiments indicated that natural resistance was not affected by the deficient diet even following prolonged feeding. After repeated infections, the control mice failed to develop resistance as they had in previous work on a different diet. Since the animals on the experimental diet harbored significantly greater numbers of worms, it suggested a breakdown in natural rather than in acquired resistance. Earlier workers using a similar deficient diet in experiments with rats showed that the controls became immune after reinfection whereas those on the deficient diet failed to develop demonstrable resistance. This discrepancy in results for the controls used in the separate studies suggested that the diet was not suitable for mice, so that a final experiment was performed after changing diets (Diet II).

The natural resistance of the mice was not affected after 11 weeks on the deficient diet (Diet II), as the number of adult worms recovered from them following infection was similar to that from controls. Mice on the deficient diet developed about the same degree of acquired resistance as the controls after two stimulating infections. Thus this deficient diet did not interfere with the operation of either natural or acquired resistance. The fact that the controls developed resistance following repeated infections supports the above view that the rat diet was unsatisfactory for this work. The findings of the final experiment with a suitable diet for mice show that the need for vitamin A in the operation of this resistance, if such need exists, is certainly not of the same magnitude for mice as demonstrated earlier for rats.

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NOTES ON *ISOETES* IN NORTH CAROLINA[1]

By A. E. Radford

Department of Botany, University of North Carolina
Chapel Hill, North Carolina

While doing field work on the vascular aquatic plants of North Carolina during the spring and summer of 1949, I collected specimens of *Isoetes* from bogs, pools, lakes, and streams in twenty-nine counties, from Tyrrell to Cherokee.

Isoetes is fairly abundant in all of the mountain counties. It is very common in the Hendersonville-Brevard area in the clear, cool lakes and pools with black mud bottoms. The leaves are broken off by muskrats and other animals and float on the surface, forming a mat so thick that it is a nuisance to swimmers

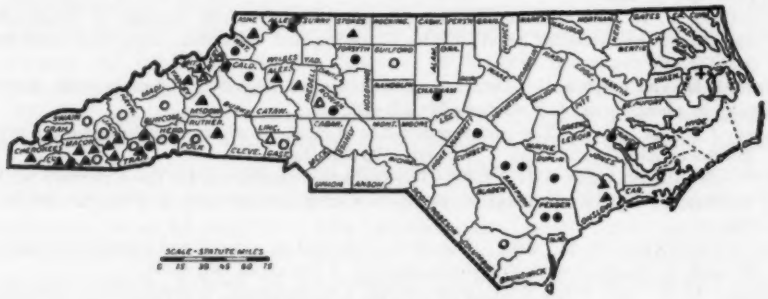

Fig. I. Distribution of *Isoetes* in North Carolina

● *Isoetes Engelmanni* A. Br. as reported in this paper.
▲ *I. Engelmanni* var. *caroliniana* A. A. Eaton as reported in this paper.
\+ *I. saccharata* Engelm. as reported in this paper.
○ *I. Engelmanni* A. Br. as reported by Blomquist and Correll.
△ *I. Engelmanni* var. *caroliniana* A. A. Eaton as reported by Blomquist and Correll.

and fishermen. In Lake Sega near Brevard and in Wolf Lake near Hendersonville, the leaves have to be cleaned from the lakes regularly. Many unsuccessful attempts have been made to exterminate it in various lakes. The Country Club Lake at Highlands is drained every winter to keep down the vegetation, but *Isoetes*, being amphibious, survives and is very abundant on the bottom of the lake.

Isoetes has a scattered distribution in the piedmont counties and is not very abundant where found. One exception is Peeler's Lake in Rowan County, a very clear, clean lake for the piedmont area, where it is plentiful. On the coastal

[1] This work was supported in part by a grant from the Carnegie Foundation for the Advancement of Teaching and in part by a scholarship from the Highlands Biological Station, Highlands, North Carolina.

plain it is found in the slow-moving streams and on the black muddy stream banks in the overflow area. No *Isoetes* was found in or near the highly acid "black water" streams and ponds of the coastal plain or mountains.

Specimens found in bogs were more robust than those growing in ponds and lakes. Some of the bog plants had leaves up to two feet long, and corms from 1½ to 2 inches in diameter.

In the Alligator River at Ft. Landing where the river opens into Albemarle Sound, within the tidal area, I found *Isoetes saccharata* Engelm. growing on *Sagittaria* rhizomes. As far as is known, this is the first record of this species in North Carolina.

Isoetes has now been reported from every mountain county of North Carolina except Madison (see Fig. I, showing data on my collections, together with those reported by Blomquist and Correll in Journ. Elisha Mitchell Sci. Soc. **56**: 104–105, 1940). Closer observation will doubtless show that *Isoetes* is more widely distributed in the coastal plain than is commonly thought. As more and more of the many farm ponds in the state become stabilized, *Isoetes* should have a greater distribution in North Carolina.

Dr. Norma E. Pfeiffer very kindly determined several specimens, among them *Isoetes saccharata* Engelm. from Tyrrell County.

Listed below are my collections of *Isoetes* from North Carolina made during the spring and summer of 1949. These specimens are deposited in the Herbarium of the University of North Carolina.

Isoetes saccharata Engelm.

Tyrrell Co. Shallow brackish water, Alligator River. Ft. Landing. #4624.

Isoetes Engelmanni A. Br.

Alleghany Co. Upper end of Lake Louise near road at Roaring Gap. #4926.
State Fish Hatchery pond at Roaring Gap. #4069.
Avery Co. Submerged in Wildcat Lake southwest of Banner Elk. #4976.
Caldwell Co. Farm pond near N.C. #18, 4 miles north of Lenoir. #4962.
Chatham Co. Bog approximately 3 miles south of Chapel Hill. #3081.
Craven Co. Core Creek near U.S. #70. #4435.
Duplin Co. Black mud on bank of Limestone Creek near N.C. #24, west of Beulaville. #5056.
Forsyth Co. Weaver's Lake near U.S. #421, west of Kernersville. #4964.
Harnett Co. Stuart Creek near U.S. #15A, 1 mile north of Erwin. #4013.
Henderson Co. Wolf Lake near Crab Creek Rd., 5 miles west of Hendersonville. #4838.
Pender Co. Sluggish stream near N.C. #53, 3 miles east of intersection of N.C. #53 and U.S. #421. #4007.
Moore's Creek near N.C. #53, west of Burgaw. #4012.
Rowan Co. Peeler's Lake near U.S. #52, north of Granite City. #4912.
Sampson Co. Bog on Six Runs Creek near U.S. #421. #4008.
Muddy bank of Six Runs Creek near N.C. #24, west of Turkey. #5059.
Transylvania Co. Eagle's Nest Camp Lake near town of Pisgah Forest on U.S. #64. #4832.
Watauga Co. Snyder's Lake at Blowing Rock. #4945.

Isoetes Engelmanni var. *carolinianа* A. A. Eaton.

Alexander Co. Black "soupy" bog in pigpen near N.C. #16, 1 mile north of Schneider's Mills. #4973.
Alleghany Co. Shallow water at Roaring Gap State Fish Hatchery. #4930.
Ashe Co. Shallow water in C. D. Bare's pond, 2 miles northeast of Glendale. #4939.
Cherokee Co. Pool in old marble quarry at Marble. #4810.
Clay Co. Bog near U.S. #64, 12 miles east of Hayesville. #4799.
Temporary roadside pool near Buck Creek bridge on U.S. #64. #4798.
Craven Co. Batchelder's Creek near U.S. #70. #4435.
Graham Co. Bog on Rock Creek near forestry road to Andrews, 1½ miles south of Robbinsville. #4813.
Iredell Co. Bog 2.2 miles east of junction of N.C. #115 and N.C. #901. #2647.
Jackson Co. Lake Sapphire east of Cashiers. #4772.
Cashiers Lake at Cashiers. #4754.
Upper Lake at High Hampton. #4820.
Macon Co. Mirror Lake at Highlands. #4788.
Lake Sequoyah at Highlands. #4786.
Country Club Lake at Highlands. #4778.
McDowell Co. State Fish Hatchery pool at Marion. #4884.
Mitchell Co. Estes Pond near N.C. #26, 1½ miles south of Spruce Pine. #4958.
Onslow Co. Black muddy creek bank near U.S. #258, 1 mile north of junction of N.C. #53 and U.S. #258. #5051.
Black mud on bank of New River near U.S. #258, northwest of Jacksonville. #5053.
Rutherford Co. McCall's Lake near N.C. #26, 2 miles east of Burke county line. #4896.
Stokes Co. Lake in Hanging Rock State Park. #3081.
Transylvania Co. Rockhouse Camp pool near U.S. #276, 4 miles south of Brevard. #4827.
Lake Sega near Brevard. #4825.
Yancey Co. Bog near N.C. #197, 1 mile north of Pensacola. #4957.

ACTINOPLANES, A NEW GENUS OF THE ACTINOMYCETALES[1]

By J. N. Couch

Department of Botany, University of North Carolina
Chapel Hill, North Carolina

Plates 10–12 and One Text Figure

In a recent paper (1949) the writer announced the discovery of sporangia and motile spores in a group of organisms related to *Streptomyces* and *Micromonospora*. Although these new forms seemed very definitely to belong to a new genus, the writer hesitated to establish one until authentic material of *Micromonospora chalceae* had been studied and until the "flagella" on the motile spores could be stained by a standard, bacterial flagella stain.

Recently there have been received from Dr. H. L. Jensen, now in Denmark, four strains "fairly representative" of *Micromonospora chalceae*, the type species of that genus, and these have proved to be quite distinct from the present forms, although closely related. Both *Actinoplanes* and *Micromonospora* grow slowly, forming a slimy-appearing, brilliantly colored mycelium on most agars. Some species or strains of *Micromonospora* which sporulate slowly or sparingly on certain agars are indistinguishable in gross appearance from some strains of *Actinoplanes*. The two genera, however, are readily distinguished. *Actinoplanes*, though a slow grower, is more vigorous than *Micromonospora*. The latter is reported to have non-septate hyphae and does not form a palisade layer at the surface of the agars, while *Actinoplanes* has septate hyphae and forms a distinct palisade layer on certain agars. They are most easily separated by their reproductive structures. In most species of *Micromonospora* the botryoid clusters of microspores are formed in such enormous quantities as to give the surface of the agar a blackish appearance. The only conidia so far observed in *Actinoplanes* are not in botryoid clusters. The most striking difference is that in *Actinoplanes* (except in coll. D.S. 22 and D.S. 34) sporangia and planospores are formed, while such structures have not been observed in *Micromonospora*. It may be of some significance that in *Micromonospora* the microspores are always formed within the medium and never in the air (Ørskov, 1923), while in *Actinoplanes* the sporangia and conidia are always formed at the surface in the air. The botryoid clusters of microspores of *Micromonospora* are shown on Plate 12.

The present genus resembles *Streptomyces* in the septate mycelial hyphae, and in the formation of palisade hyphae within the agar in at least two species of *Streptomyces* and in all species of *Actinoplanes*. In *Streptomyces* the conidia are formed in chains which in some species are arranged in spirals. It is of interest to note that in certain species of *Actinoplanes* the mature spores are usually

[1] This work was made possible by a grant from the Carnegie Foundation for the Advancement of Teaching, which enabled the writer to employ Mrs. Alice R. Giles to do the culture work.

arranged in spirals within the sporangia. The conidia in *Streptomyces* are about the same size and shape as are the planospores of *Actinoplanes*. The most striking differences between these two genera are the production in *Streptomyces* of a conspicuous aerial mycelium with abundant conidia while in *Actinoplanes* aerial mycelium is lacking except in one atypical species (D.S. 22 and 34) and conidia are few or lacking and the production in the latter of sporangia and swimming spores.

Certain species belonging to this genus or related to it have apparently been seen and described in part previously. At the Mycological Society meetings in New York in 1949, after seeing the writer's lantern slides and hearing the talk, Dr. J. S. Karling remarked that he had collected these forms many times, and that Miss Johanson had described a species belonging here under the name *Myceliochytrium fulgens* (1945). Miss Johanson interpreted this new genus as a polycentric chytrid and included it tentatively in the Cladochytriaceae. It has now been generally accepted that the structure and action of the motile cells are of the greatest importance in determining relationships in these lower fungi. Miss Johanson describes the swimming spores of her fungus as being posteriorly uniflagellate and structurally similar to the swimming spores of most known chytrids. However, as reported by the writer (1949) the motile spores of the present group are unlike those of any fungus but rather more like those found in certain bacteria. Thus the genus *Actinoplanes* differs from *Myceliochytrium* in the most critical feature. Now it is possible that Johanson's organism is correctly described and that it is a true chytrid with posteriorly uniflagellate swimming spores. If this is the case, her organism would be a true member of the lower Phycomycetes, and the rather striking similarities in mycelium and sporangia between her genus and the forms treated here would merely be another example of parallel development in two distantly related groups. It is more likely however that her description was drawn up from mixed material and that the swimming spores which she described as posteriorly uniflagellate and structurally similar to those of the chytrids actually belonged to a small true chytrid which was being carried on in her leaf cultures with the *Actinomyces*-like forms. There seems little doubt that the organisms described in the present paper were seen and partly described by Miss Johanson. The question, therefore, arises as to what name to use for these new forms? Since the name *Myceliochytrium* appears to apply to mixed material (a fungus and an Actinomycete), it can hardly be considered as valid. If on the other hand her material was not mixed, then the name *Myceliochytrium* stands as valid but applies to an organism with posteriorly uniflagellate swimming spores and hence quite distinct from the forms treated here.

The character of the flagella on the swimming spores is still uncertain. Dark-field observations have been repeated, using a Zeiss cardioid condenser, a ×35 oil immersion objective, and an arc lamp. The swimming spores were put in a 0.25 per cent solution of Methocel of the 15 cps viscosity recommended by Pijper (1947) as most suitable on the whole for viewing the flagella of bacteria. The spores were slowed down somewhat but no flagella were visible.

Stained preparations gave conflicting results. If killed with the fumes of 1 per cent osmic acid solution and stained with gentian violet a small percentage of the spores showed structures which might be flagella, one to a spore as reported in the former paper. Mr. W. J. Koch, graduate student in this department, using a bacterial flagella stain (Lee's method) has been able to demonstrate several "flagella" on each spore (Plate 10, fig. 1). These structures appear to be attached at the anterior end of the spore, perhaps bending backward as the spore is in motion. By using this same method, Mr. Koch was able to make very nice preparations showing typical flagella on certain rod-shaped bacteria. The assumption that these appendages on the spores of *Actinoplanes* are the organs of locomotion is of course open to doubt. From studies so far, it appears that the motile cells of these new forms are unlike those of any of the Phycomycetes but rather more like the bacteria.

Up to the present, thirteen apparently new forms have been isolated from soil collections. Since these do not fall into any known genus of the Actinomycetales, a new genus and the species upon which it is based are being described herein. In a later paper the remaining species will be described.

ACTINOPLANES NEW GENUS

Mycelium formed in water on a variety of plant and animal parts, also in various nutrient solutions and on various agars, usually brilliantly colored, pink, orange, etc., rarely nearly hyaline or dark brown. Aerial mycelium lacking as a rule, formed in certain species and then much as in *Streptomyces*. Surface on agar smooth or bumpy or convoluted, usually moist and shiny, rarely pulverulent. On boiled leaves in water, forming a very inconspicuous mycelium which grows in tufts and fringes around the edge of the leaf and also in tufts over the upper surface, giving the leaf a speckled appearance. Hyphae delicate, branched, sparingly septate; on agar, of two distinct forms, horizontal and vertical, the latter in some species making a compact palisade layer at the surface. Sporangia present usually abundant, borne at the surface on the palisade hyphae in contact with air, spherical, subspherical, or irregular in shape. Spores usually arranged in coils in the sporangia, discharged at maturity when flooded with water, spherical to cylindrical, small, with a single, minute globule; swimming vigorously or in some species non-motile. Flagella not certainly visible in living material, staining as in certain bacteria, several apparently attached at the apical end. Conidia formed in some species but not in botryoid clusters as in *Micromonospora*.

Aerobic; Gram positive and liquefying gelatin (Knox).

Sapropytic in a variety of soils and perhaps also in aquatic habitats.

Actinoplanes philippinensis n. sp.

Plates 10 and 11 and Text Fig. 1

On boiled leaves of grass (*Paspalum*) in water, forming a very delicate, hyaline to pinkish mycelium which grows in tufts and fringes around the entire edge of the piece of leaf and also in places over the upper surface, giving the leaf a speck-

led appearance; hyphae of uneven diameter, 0.5–1.5μ thick, branched, sparingly septate. Sporangia formed abundantly on grass after about ten days, also formed on certain agars, spherical to subspherical, usually on unbranched stalks, 8.4–27μ thick, most about 16μ thick; spores at maturity arranged in coils, about 1–1.2μ thick and with a single hyaline to yellowish globule, discharged through a pore or by the partial dissolution of the sporangial wall and swimming vigorously; flagella as described for the genus. Conidia formed in compact clusters of 12–20 on blocks of nutrient agar transferred to water, about 2 x 8–12μ, bent and with 5–7 constrictions.

On potato dextrose agar, growth vigorous, 1.8–2.2 cm. in diameter in 6–8 weeks (temp. 16–19°C.); margin 2–3 mm. wide, flat; central area elevated 2–3 mm. above the agar, convoluted, very tough in texture, composed of densely compacted, intertwined hyphae; color darker than on other agars, at first near

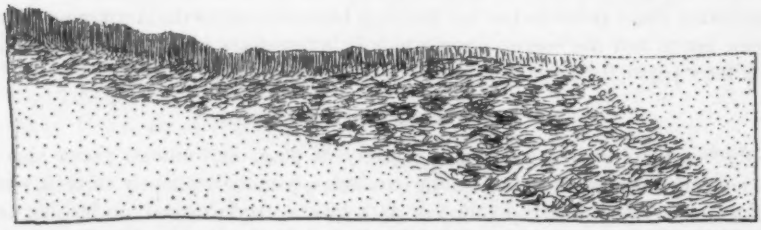

FIG. 1. Diagrammatic sketch of section of *Actinoplanes philippinensis* on Czapek's agar, Aug. 29, 1949. ×8 approx. The vertical lines at top represent palisade hyphae (bearing sporangia at left); the irregular lines represent the intramatrical, entangled, horizontal hyphae; the dotted area, agar.

apricot-orange,[2] then mahogany red, chestnut, or cinnamon-rufous; mycelium giving off a diffusible pigment which darkens the surrounding agar. Palisade hyphae distinct in the non-convoluted region, forming a lattice work with the thinner, horizontal threads, becoming less distinct in older parts, many of the upright threads forming at the tip one sporangium each.

On nutrient agar, growth fair, about 1 cm. in diameter in 6–7 weeks (temp. 16–19°C.); margin 2–4 mm. wide, flat; center slightly elevated; color orange to cinnamon-rufous. Palisade hyphae not distinct.

On Krainsky's dextrose asparagin agar, growth vigorous, 1.6–2 cm. in diameter in 6–9 weeks (temp. 16–19°C.); margin narrow, flat; center up to 2 mm. high, convoluted; color ferruginous. Palisade hyphae distinct in flat area; sporangia formed over part of the palisades.

On Czapek's agar (1.5% agar), growth poor, 0.3–0.4 cm. in diameter after 7–9 weeks (temp. 16–19°C.), mostly submerged; center slightly elevated, rough; color cinnamon to tawny. Palisade hyphae very distinct, rarely branched, thicker than the much-branched, coiled and twisted hyphae; many sporangia formed in certain areas of the palisade hyphae.

[2] All colors are from Ridgway's *Color Standards and Nomenclature*.

On Czapek's agar with glycerine in place of sucrose, growth only slightly better than on Czapek's agar, 0.5 cm. in diameter in 7–8 weeks (temp. 16–19°C.); surface elevated, convoluted, no flat margin; color clay, cinnamon-rufous to darker; mycelium giving off a diffusible pigment which darkens agar as in P.D.A. No distinct unbranched palisade hyphae, upper layer thick, composed of upright but not vertical, branched, entangled hyphae. Upright hyphae thicker than the more entangled horizontal ones. No sporangia seen.

On Czapek's broth in flask, growth good, many floating and submerged patches after 6 weeks (temp. 20–23°C.), semi-globose, varying in size from almost invisible to 2 mm. thick; color bittersweet orange; composed of palisade hyphae on upper surface and much-entangled, branched and twisted hyphae below. Many sporangia formed on the palisade hyphae.

From a very small quantity of soil (coll. no. P15) sent to me by Lieutenant W. Lane Barksdale from the Philippine Islands in 1945; first seen in February 1948.

This species is characterized by its comparatively vigorous growth on dextrose asparagin and potato dextrose agars and its rather poor growth on nutrient agar, Czapek's, and Czapek's agar with glycerine replacing sucrose, at temperatures of 16–19° C., its deep brownish color on the first two agars after 30 days, its larger sporangia, and by its production, in potato dextrose and in Czapek's agar with glycerine replacing sucrose, of a diffusible dark brown pigment. In fact, of the thirteen strains or species which we have in pure culture only two produce such a pigment.

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EXPLANATION OF PLATES

PLATE 10

FIG. 1. Planospores of *Actinoplanes philippinensis* No. P 5 killed with fumes of osmic acid and stained with Lee's bacterial flagella stain by W. J. Koch. ×2425. Photograph by Koch.

FIG. 2. *A. philippinensis*. Sporangia on edge of dandelion leaf. Note dots in spores. One sporangium discharging spores. ×700.

FIG. 3. Same material as in Fig. 2. Four sporangia opening, three with spores in chains, one with spores in spirals. ×700.

FIG. 4. Same material. Spores in chains or coils, the masses spreading out. ×700.

FIG. 5. Same material. Several sporangia in various stages of dehiscence, the one on the surface of the leaf with spores distinct on its lower side and densely packed and indistinct on the upper. Hyphae distinct on surface of the leaf. ×700.

PLATE 11

FIG. 1. ***Actinoplanes philippinensis***. Thin, crushed section of palisade hyphae showing a single sporangium on the end of most of the threads (on Czapek's agar). ×300.

FIG. 2. Same, but showing a larger and thicker section with many palisade hyphae and many sporangia. ×150.

PLATE 12

FIG. 1. ***Micromonospora*** (near ***chalceae***). Chapel Hill No. 3. Showing large clusters of microspores in darker areas in upper part, one broken off on left. ×400.

FIG. 2. Same but several clusters of microspores spread out by crushing. ×1400.

FIG. 3. ***Micromonospora*** sp. Chapel Hill No. 4. Microspores in loose clusters. ×700.

FIG. 4. Small cluster of same showing formation of spores. ×1400.

PLATE 10

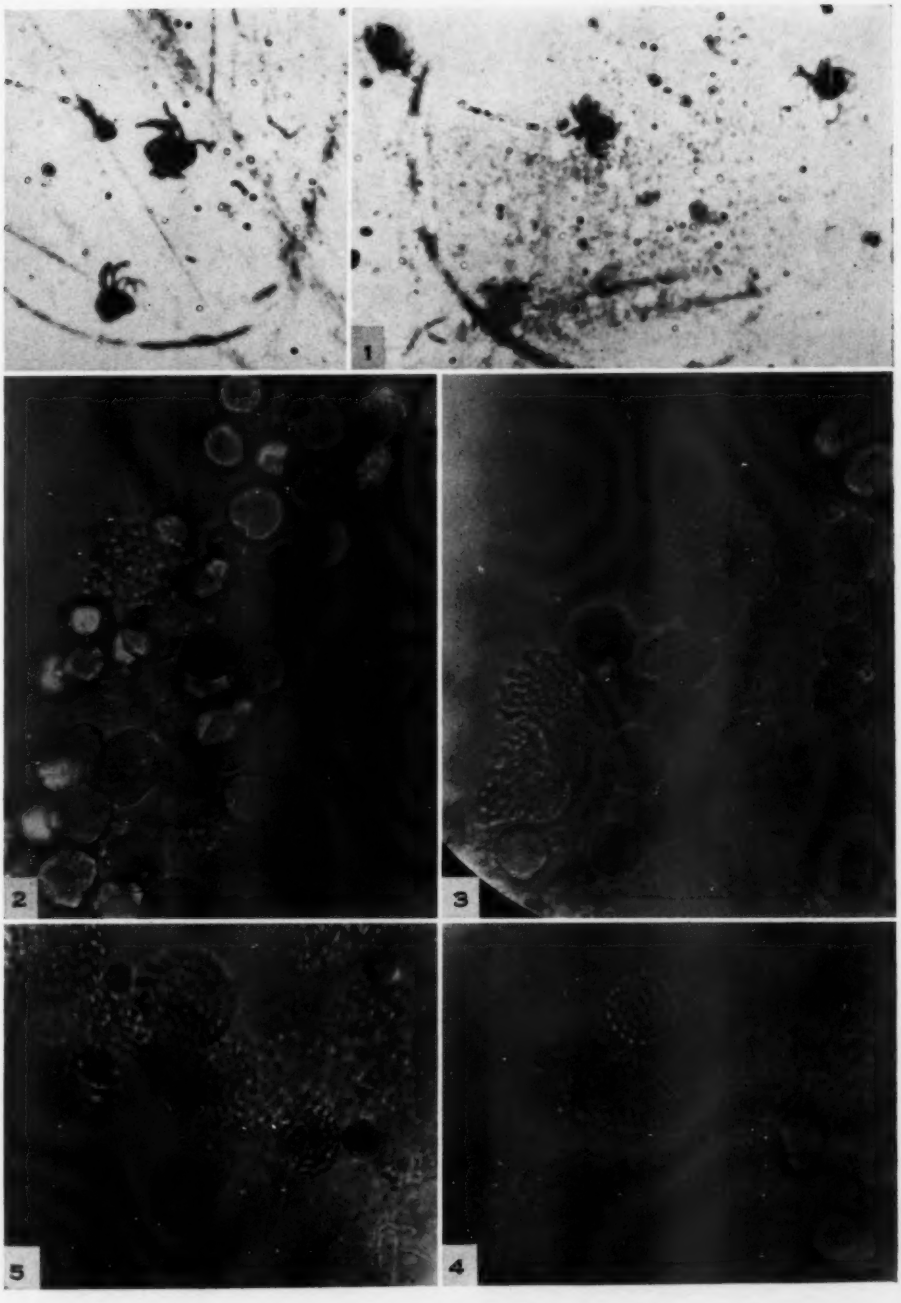


PLATE 11

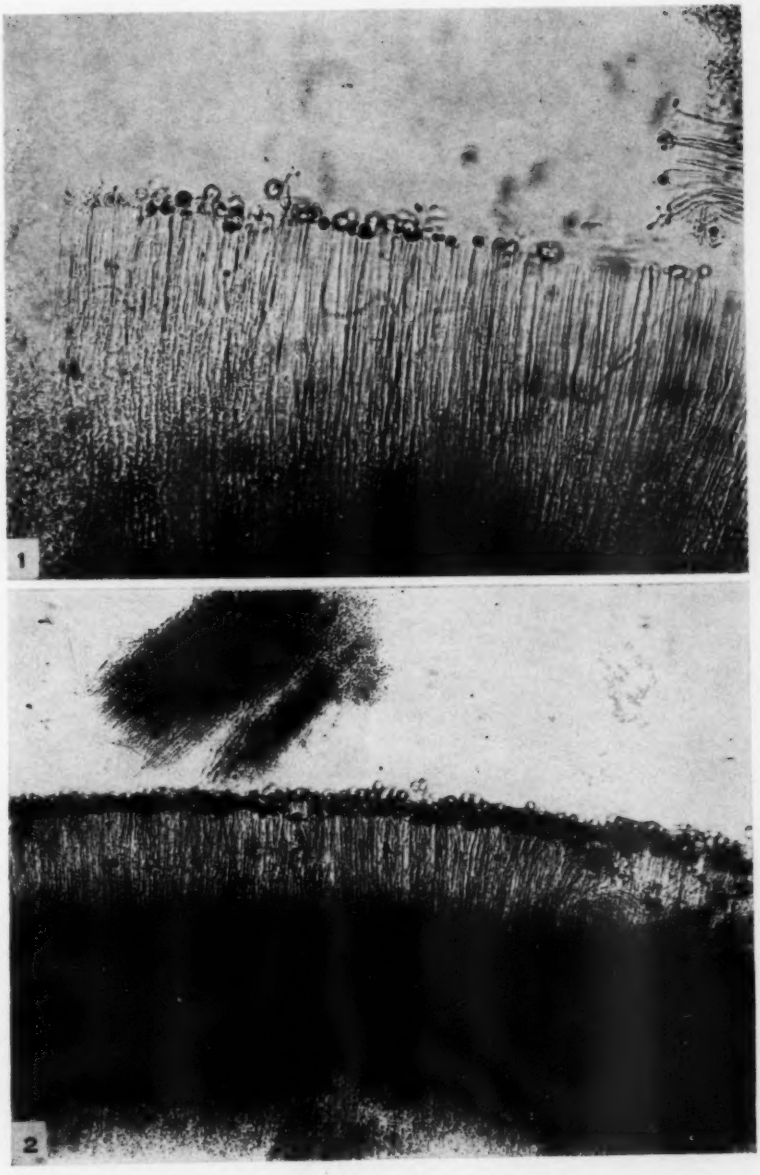
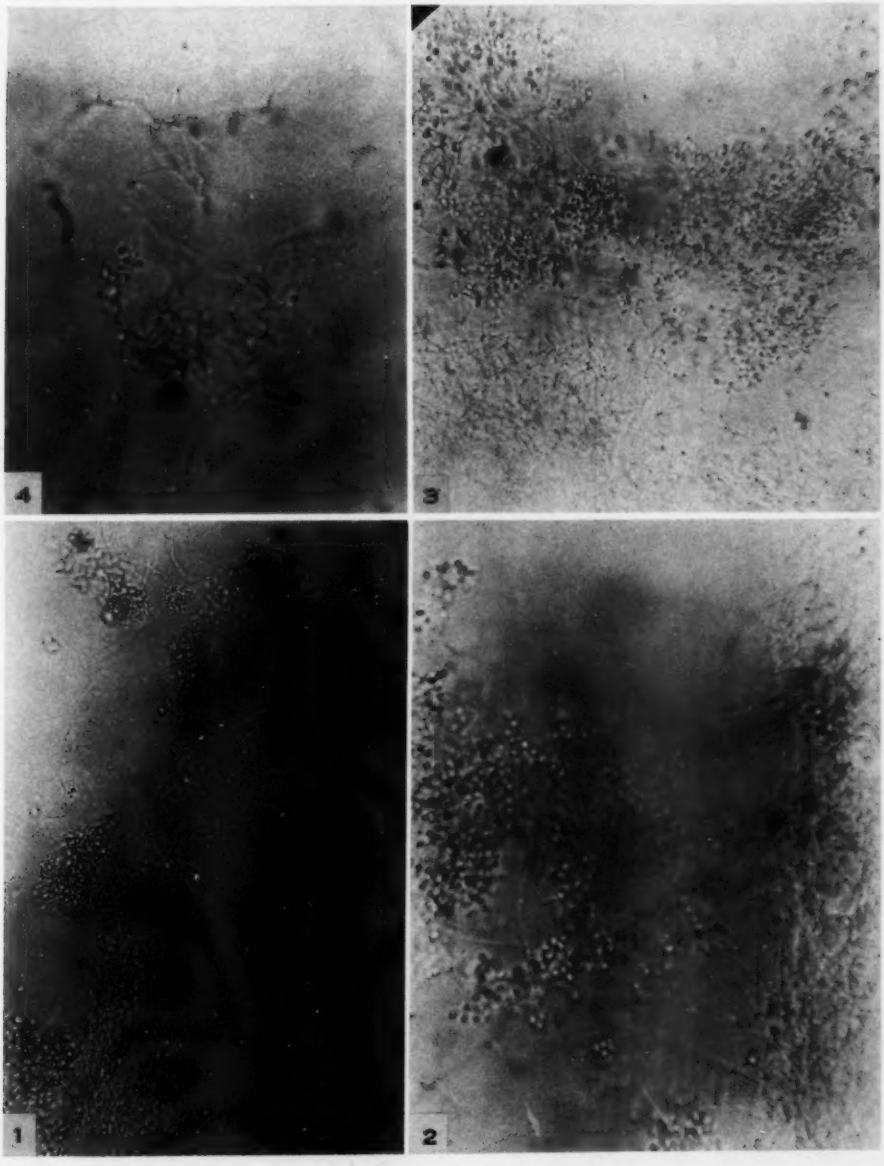


PLATE 12



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